EFFICACY OF LUGOL'S IODINE IN THE EVALUATION OF VOCAL CORD NEOPLASM

A DISSERTATION SUBMITTED IN PARTIAL FULFILLMENT OF **M.S BRANCH –IV**

(OTORHINOLARYNGOLOGY) EXAMINATION OF THE TAMILNADU DR.MGR.

MEDICAL UNIVERSITY TO BE HELD IN **APRIL 2013**

CERTIFICATE

This is to certify that the dissertation entitled **'EFFICACY OF LUGOL'S IODINE IN THE EVALUATION OF VOCAL CORD NEOPLASM'** is a bonafide original work of **Dr Anand VK,** submitted in partial fulfillment of the rules and regulations for the MS Branch IV, Otorhinolaryngology examination of The Tamil Nadu Dr. M.G.R Medical University to be held in April 2013.

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CONTENTS

INTRODUCTION

Cancer of the larynx is the second most common malignancy of the upper aerodigestive tract (UADT). Even though large varieties of malignancies are reported in the larynx, 90%[1, 2] of them are Squamous Cell Carcinoma (SCC) which arises from the epithelial lining of the larynx. The most common site of laryngeal carcinoma is the glottis[3].

About 90% of malignant tumors of the larynx are carcinomas that often develop from premalignant lesions[4]. Therefore, early detection and prompt treatment should thus prevent the development of invasive cancer requiring more debilitating surgical resection[5]. It is very difficult to predict accurately which lesions will progress to invasive malignancy based only on clinical appearance.

Studies have proven that the clinical appearance bears little correlation with the underlying pathology. What makes decision making difficult is that simple hyperplasia, dysplasia, and or carcinoma can all coexist in same lesion. Even, stroboscopy has not proved to be reliable method of determining the presence of malignancy or depth of invasion. At present there is no standard test to identify benign lesions from premalignant or malignant ones.

Many attempts have been done to differentiate a premalignant from a malignant lesion intra operatively. This includes the application of vital dyes like toluidine blue and methylene blue, contact endoscopy, induced autofluorescence using 5-ALA (5-aminolevulinic acid), compact endoscopy (ie combined use of autofluorescence and contact endoscopy) and Optical

Coherence Tomography (OCT). But the wide spread use of these techniques were limited by cost factors and limited sensitivity and specificity.

In this study a novel approach has been tried for differentiating benign, pre malignant and malignant lesions in the vocal cords, intra operatively by using the vital dye Lugol's iodine, which is widely used in the oral cavity for the same purpose[6]. Lugol's iodine has also been used in oesophagoscopy as safe, inexpensive, simple and rapid to perform, easy to interpret and highly sensitive adjuvant for all clinically important squamous neoplasia. Evidence shows that visual inspection methods using Lugol's iodine has more sensitivity, visual inspection allows an immediate result and, when appropriate, may be immediately followed by therapy, the so called "screen-and-treat" approach[7]. Its effectiveness as a screening strategy to decrease cervical cancer mortality and to increase life years is also well studied. Hence Lugol's iodine is a good choice as a visual staining agent to identify/exclude cancerous and precancerous lesions in resource-constrained region[8]. This same principle is being applied in the detection of pre malignant and malignant lesions of the glottis region.

AIMS & OBJECTIVES

Objective

To evaluate a commonly available, easily applicable and cost effective method to diagnose the presence of pre malignant and malignant vocal cord neoplasm.

Aim

1. To observe the staining property of Lugol's iodine in various vocal cord lesions.

2. To assess the reliability of Lugol's iodine in the evaluation of pre malignant and malignant vocal cord lesions.

The hypothesis is that Lugol's iodine "stains" normal epithelium and benign lesions where as

pre malignant and malignant lesions remain unstained.

REVIEW OF LITERATURE

Cancer of the larynx is the second most common malignancy of the upper aerodigestive tract (UADT)[1]. This accounts for approximately 1.7% of all new cancer diagnosis, 25% of all head and neck malignancies and in 90% of cases it is squamous cell carcinoma[2].

In India, laryngeal carcinoma constitutes 2.63% of all body cancers, ten times more common in males than females (4.79% vs 0.47%) with an incidence of 3.29 new cases in males and 0.42 new cases in females for one lakh population[9]. There seems to be a tendency to be mainly a disease of the middle aged men with a peak incidence in the seventh decade. Women are affected in a comparatively younger age with a peak incidence at less than sixty years[10].

Although a large variety of malignancies are reported in the larynx, 85-95 percentage of laryngeal malignancies are squamous cell carcinoma (SCC), arising from the epithelial lining of the larynx[11]. Classically laryngeal carcinoma is divided by sub sites into supra glottic, glottic and sub glottic cancers. The most common site for laryngeal squamous cell carcinoma is the glottic larynx[3].

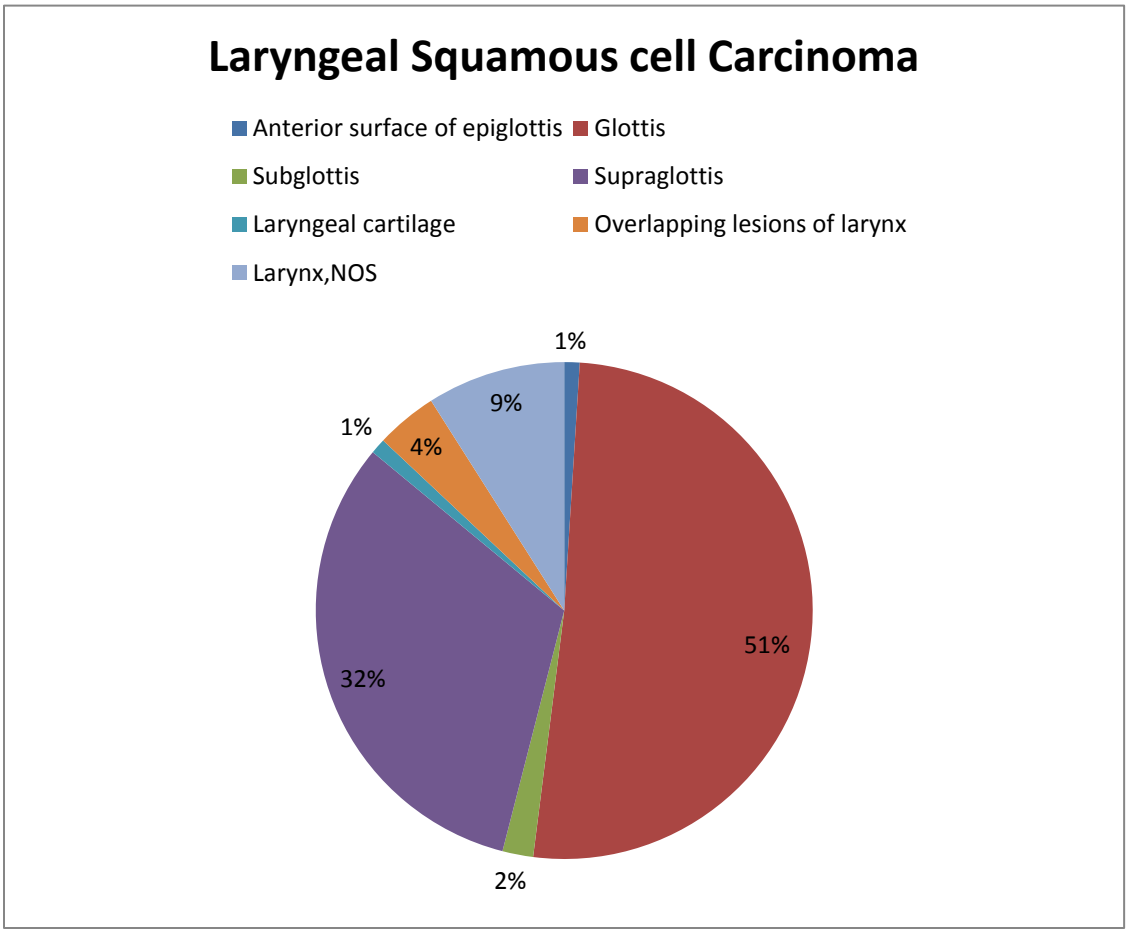


Fig. 1 Distribution of laryngeal squamous cell carcinoma[3]

Aetiology of laryngeal carcinoma

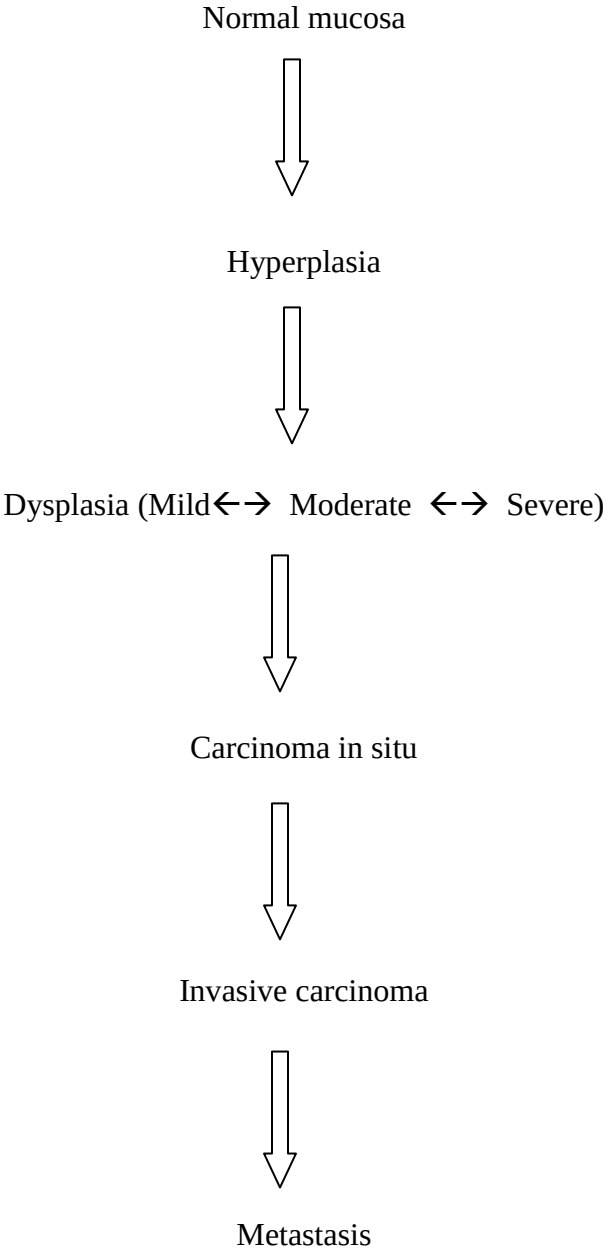
Smoking, alcohol, chemical exposure, laryngopharyngeal reflux, viral etiology and diet are considered as the risk factors for laryngeal cancer. Squamous cell carcinoma of the larynx is strongly associated with the use of tobacco and alcohol. They are the two strongest aetiological

factors for the development of Head and Neck Squamous Cell Carcinoma (HNSCC), both independently and synergistically[12, 13.] Tobacco has been identified as the main causative agent in laryngeal cancer, with up to 98% of patients being smokers[14]. In non smokers, alcohol may increase the relative risk of laryngeal carcinoma by five fold.

Nickel and chromate refining workers have an increased incidence of laryngeal squamous cell carcinoma[15]. Chronic laryngeal inflammation caused by acid reflux may be a carcinogenic co factor similar to Barrett's oesophagus. Gastric acid reflux has been established as a risk factor for laryngeal carcinoma. Recently alkaline reflux has been identified as a laryngeal carcinogen by Gilli and colleagues[16]. They identified a higher number of patients with laryngo pharyngeal cancer associated with achlorhydria and presumed alkaline reflux after gastric resection. They also identified that, in patients with laryngeal carcinoma with an intact gastric acid secreting mechanism, 81% showed abnormal acid reflux on 24 hour pH monitoring[16]. Human Papilloma Virus (HPV) subtypes 16 and 18 found to be closely related to laryngeal carcinoma and it is identified in 40% of cases[17]. HPV is an independent risk factor for laryngeal carcinoma. Poor nutritional status is associated with increased relative risk of laryngeal carcinoma. Fruits and vegetables are found to have a protective effect, which may be attributed to their high Vitamin A and Vitamin C content[18].

The multiple aetiological factors act on the various levels of the molecular structure of the epithelial cell lining of larynx and act as carcinogens. So a series of events occur and there is a progression of normal mucosa to pre malignant lesions and then to invasive disease[19].

Progression of normal epithelium to pre malignant and to invasive disease in Squamous Cell Carcinoma.[19]



Anatomy, Embryology and Histology of vocal cords

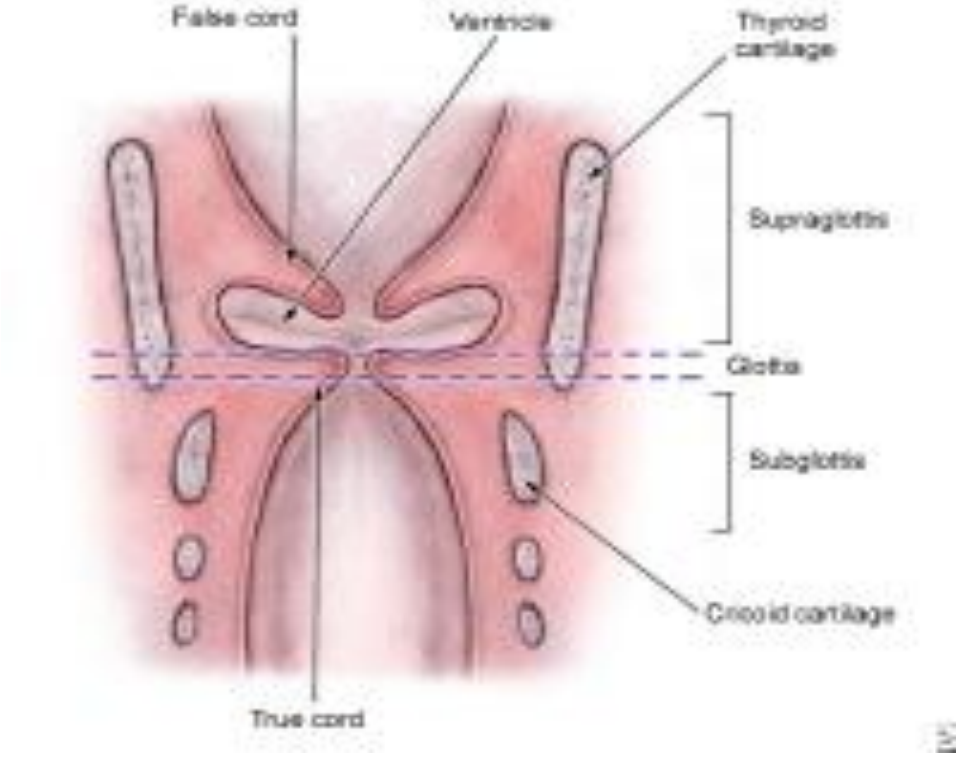


Fig.2 Anatomy & Subdivisions of the larynx

Embryology

The larynx is divided into three regions or sites: supra glottis, glottis and sub glottis. This division reflects the embryologic structure of the larynx and the anatomical barriers to spread of laryngeal cancer. The pattern of spread of tumours within the larynx is guided by the ligaments, connective tissue membranes, cartilages of the larynx that contain the spread of tumour, as well as by the soft tissue spaces within the larynx, that act as pathways within and outside of the

larynx. The characteristic tumour pattern can be explained by the embryologic development of the larynx. The supra glottis is derived from the buccopharyngeal primordium, which develops from the third and fourth branchial arches. The glottis and sub glottis are developed from the tracheobronchial primordium. Due to this reason the larynx has a dual blood supply and lymphatic drainage. The supra glottis is supplied by the superior laryngeal arteries and its lymphatic drainage follow these vessels into the carotid sheath into the deep cervical nodes in level 2 and level 3. The glottis and sub glottis are supplied by the inferior laryngeal arteries, and lymphatic drainage from these regions follow these arteries to drain into pre laryngeal and pre tracheal nodes, and finally drains into deep cervical nodes in level 4 [20]. The glottic region is formed by the paired structures that fuse in the midline. So the lymphatics drain unilaterally. The vocal folds have sparse lymphatics. This explains the lower incidence of lymphatic metastasis in glottis squamous cell carcinoma. But the supra glottis is formed without a midline union, its lymphatics drain bilaterally and this causes the increased likelihood of bilateral lymphatic metastasis from supra glottic carcinoma[21, 22].

Anatomy

The larynx is divided systematically into supra glottis, glottis and sub glottis. This division reflects the embryologic structure and the anatomic barriers to spread of laryngeal cancer. The supra glottis is composed of the supra hyoid and infra hyoid epiglottis (both the lingual and the laryngeal surfaces), aryepiglottic folds (laryngeal surface only), arytenoids and the ventricular bands (false cords)[23]. The inferior limit of supra glottis is a horizontal plane through the lateral margin of the ventricle at its junction with the superior surface of the true vocal cords. The glottis consists of bilateral true vocal cords including the anterior and posterior

commissures. The inferior surface of the glottis is a horizontal plane 1cm inferior to the inferior limit of the supraglottis [23.] The sub glottis extends from the inferior limit of the glottis to the inferior edge of the cricoid cartilage. Subglottis is not divided into any further subsites[23].

Histology

The mucosal lining of the larynx differs in the three regions. The epithelium of the supra glottis is predominantly of the pseudo stratified columnar type, except at the edges of the aryepiglottic folds and the lateral borders of the epiglottis, which is stratified squamous epithelium. The true vocal cords have a unique structure: non keratinized stratified squamous epithelium, which covers a three layered lamina propria. The lamina propria is composed of superficial, intermediate and deep layers. The intermediate and deep layers of lamina propria form the vocal ligament. The subglottis is lined by pseudo stratified columnar epithelium[24].

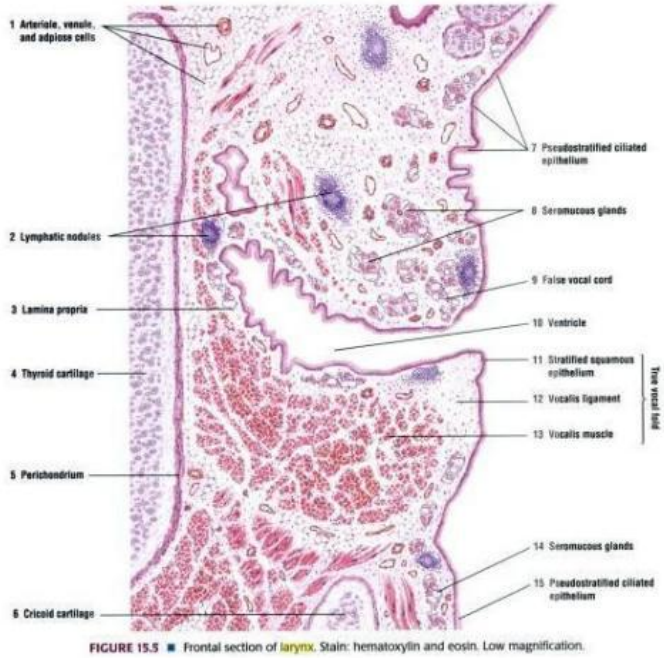


Fig.3 Histology of the vocal cords.

Clinical evaluation of Suspected Vocal Cord Pathology:

History and Presenting Complaints: Hoarseness is a non specific symptom that can result from a variety of disease processes ranging from a benign polyp to potentially life threatening carcinoma. It can also be a manifestation of systemic disease that can affect the larynx. Any patient with hoarseness of more than two weeks should undergo a proper vocal cord assessment. A proper evaluation includes a thorough history, physical examination and vocal cord visualisation including indirect laryngoscopic examination and endoscopic assessment of vocal cords.

Obtaining an accurate history is of utmost importance, hence a detailed history about onset, duration, and severity of dysphonia should be enquired. Other symptoms like stridor, dyspnoea, dysphagia, odynophagia etc should be noted. History of voice abuse and risk factors like smoking and alcohol intake is essential. Past history of any laryngeal surgeries and systemic illness is to be recorded.

Clinical Examination: As with any oto-laryngological examination, a complete head and neck examination is essential especially to evaluate the neck nodes. The indirect mirror exam is the initial procedure used to visualize the larynx. It is quick, inexpensive and only requires only a mirror and external light source. In this gross abnormalities may be detected quickly, but minor lesion may be missed.

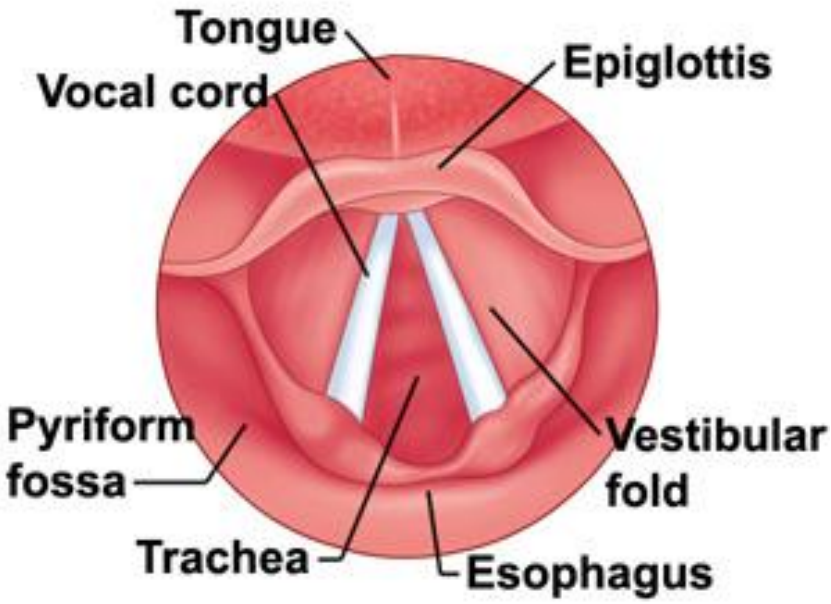


Fig.4 Appearance of larynx on indirect laryngoscopic examination

Rigid laryngeal endoscopy is performed using 70 or 90 degree endoscopes passing through the mouth to obtain images of the larynx and pharynx. These are the highest quality images obtainable and offer excellent magnification.

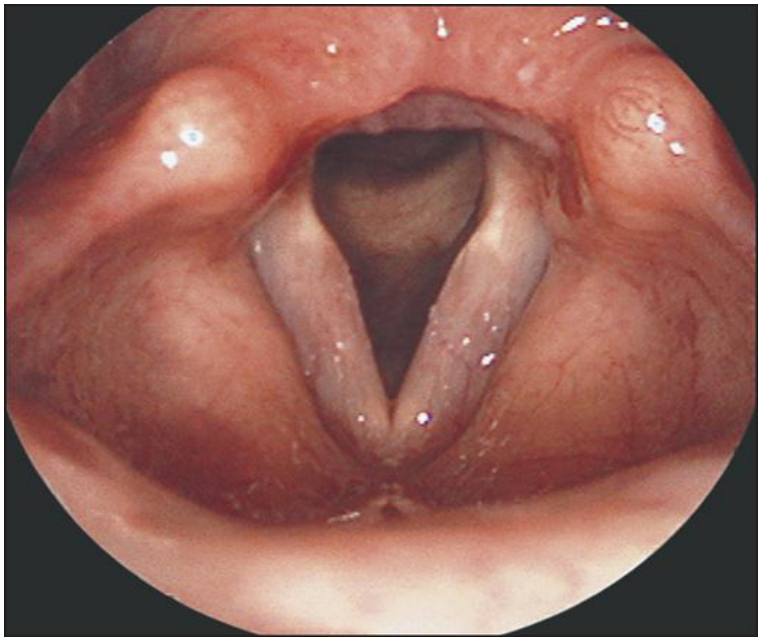


Fig.5 Fibrolaryngoscopy picture of the vocal cords

Grabas CS et al[25] in their study on the relevance of mirror examination on larynx commented that 84% of the indirect laryngoscopies could be used to give a reasonable examination of the larynx, but it is not adequate, and fibrolaryngoscopy is a good alternative and a more reliable examination. Disadvantages include the larynx not being in physiologic phonation position, hyper reflexive gag, uncooperative patient and nonvisualisation of hidden areas of larynx like subglottis, ventricles, laryngeal surface of epiglottis etc. In a study by Barker et al [26] the diagnostic accuracy and examination, the success rates of indirect laryngoscopy versus a rigid rod examination were assessed and compared. 52% of mirror examinations were successful as were 83% of rod exams. They conclude that the mirror is a

useful screening tool so long as strict examination criteria are used. The rigid rod endoscopy adds further diagnostic information in those patients who cannot be evaluated with a mirror.

The flexible laryngoscope is probably the instrument that most otolaryngologists use in the evaluation of the dysphonic patient. It is the only method that allows examination of the nasopharynx, palate, larynx, and pharynx in a near physiologic position. It can be performed relatively easily in patients with hyper-responsive gags and pediatric patients. This is the most commonly used office procedure for the evaluation of vocal cord pathology.

Videostrobolaryngoscopy (VSL) allows for dynamic assessment of the vocal folds. The features most helpful in the diagnostic process include the vocal fold closure pattern, vocal fold vibratory pattern, and the mucosal wave of each vocal fold during phonation. It is an excellent tool in the assessment of vocal cord mobility, mucosal wave pattern and detecting early lesions of the vocal cords.

Fig.6 Stroboscopic images showing vocal fold vibratory pattern and the mucosal wave

Benign lesions of the vocal cord

Benign vocal cord lesions are non cancerous growths of abnormal tissue in the vocal folds. They are commonly referred to as Benign Vocal Fold Lesions (BVFLs), according to the new nomenclature system, proposed by Clark A Rosen et al [27]. According to this classification, nine distinct vocal fold lesions are mentioned.

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1. Vocal fold nodules.
2. Vocal fold polyps
3. Pseudocysts

4. Vocal fold cysts- subepithelial
5. Vocal fold cysts- ligamental
6. Non specific vocal fold lesions (NSVFL)
7. Vocal fold fibrous mass- subepithelial
8. Vocal fold fibrous mass- ligamental
9. Reactive vocal fold lesions.

Various factors have been attributed to the aetiology of benign vocal cold lesions. The most important of which is chronic voice abuse. Others are acute vocal misuse, trauma resulting from infection and inflammation caused by Gastro Esophageal Reflux Disease (GERD). The other factors which contribute to the chronic irritation of the larynx include post nasal drip caused by allergic rhinitis or chronic sinusitis, exposure to chemical irritants such as that from tobacco or substance abuse, pulmonary disease which may lead to poor breath support during speech, cough variant asthma, poor vocal hygiene and systemic diseases like hypothyroidism.

Various benign vocal fold lesions (BVFL) include

Vocal fold polyps: A true focal fold polyp is a benign swelling of greater than 3mm that arises from the free edge of the vocal fold[28]. It is usually solitary, but can rarely affect both vocal cords and can be pedunculated or sessile. It is known that polyps are the most common lesion of the vocal cord that causes hoarseness. It affects men more than women and most commonly seen in smokers between the age of 30 to 50 years [29, 30]. Phono trauma is the most common cause for polyp formation. Vocal fold polyp is exophytic and often translucent or haemorrhagic. The

overlying mucosa is usually thin and atrophic. Histology shows a gelatinous substance without encapsulation within the stratified squamous epithelium [25]. Most polyps do not resolve or become smaller with voice rest or voice therapy and they need excision under general anaesthesia.

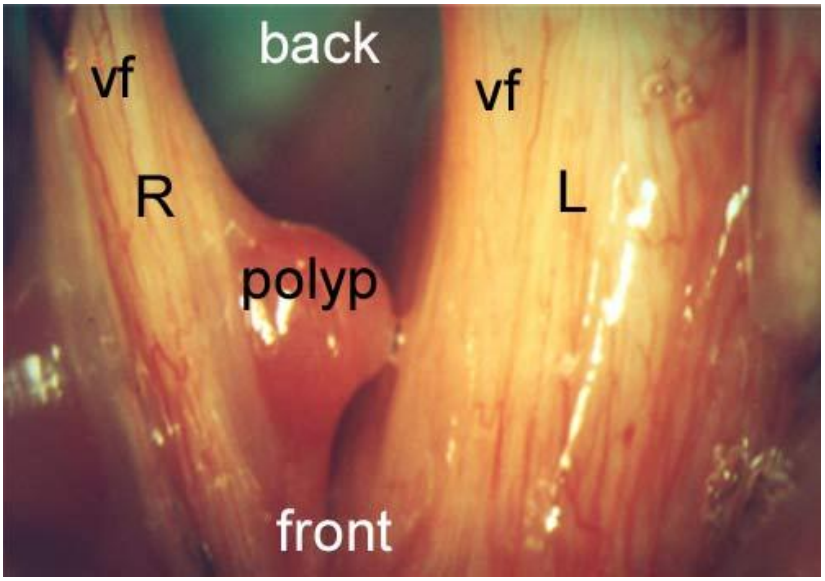

Fig.7 Polyp of the right vocal cord.

Vocal fold nodules: Vocal nodules are bilateral small swellings (less than 3mm in diameter) that develop on the free edge of the vocal fold at the mid membranous portion. The aetiology of voice abuse is traditionally believed to be voice abuse. Vocal fold nodules are characterised histologically by thickening of the epithelium and underlying inflammation [27]. This may be associated with microwebs in the anterior commissure in some cases. Its incidence is found to be 25% in children[31] and is 6% in adults with persistent hoarseness of voice. But higher percentage

is found in teachers and singers[32]. In children, they are most common in boys, than girls, but in adults it is most common in young ladies especially below the age of 30 years [27]. A key stroboscopic feature for vocal nodule is normal or minimal impairment of the vibratory properties of the mucosa [25]. A focal nodule respond well to voice therapy and voice rest and these lesions are usually treated non operatively [25].

Fig.8 Bilateral Vocal cord nodules

Pseudocyst: It is a sub epithelial lesion associated with chronic glottal incompetence, eg: vocal scar, paresis or paralysis. It is usually unilateral. The lesion is very superficial in nature. The lesion is very clear with blister appearance that contains a semisolid fluid underneath thinned epithelium without encapsulation [25]. The vibratory properties of the vocal fold mucosa are

normal or minimally reduced. Pseudo cyst does not respond to conservative therapy. The treatment is surgical removal with correction of underlying glottal incompetence.

Vocal fold cysts: Vocal fold cysts (VFC) may be within the sub epithelium or in the ligament. It is usually unilateral. Significant reduction in vibratory function of the mucosa is noted in stroboscopy. Vocal fold cysts do not resolve with voice therapy. Microlaryngoscopic surgery is needed and it is confirmed intra operatively by the presence of an encapsulated lesion.

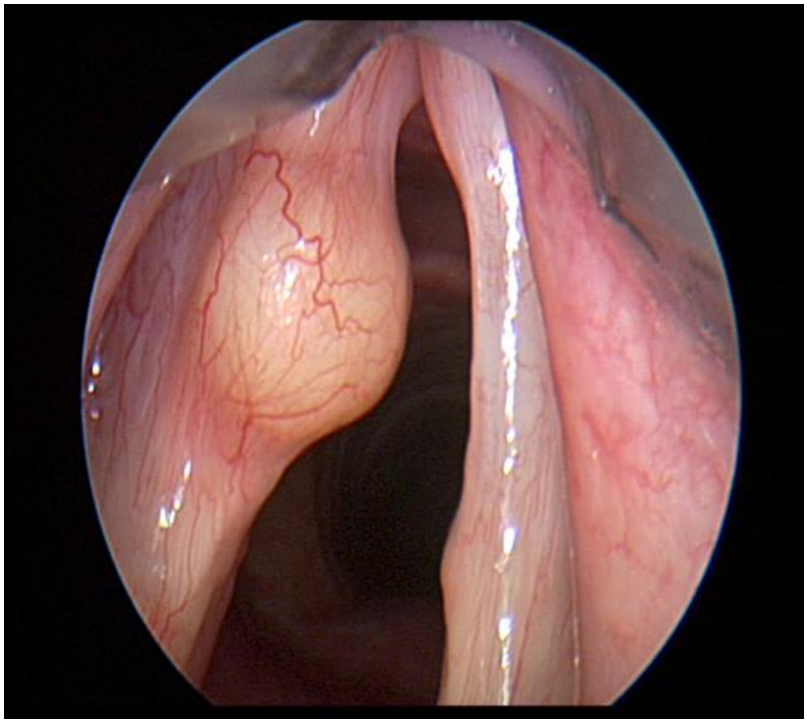

Fig.9 Vocal Fold Cyst

Vocal fold fibrous mass: This lesion is typically amorphous and without encapsulation. It has a focal point within the vocal fold (seen stroboscopically) and can be palpated intra operatively.

Fibrous mass can be seen in the sub epithelium or in the ligament (FM-SE or FM-lig). Fibrous mass causes a significant diminution of vibratory function of mucosa. Histologically it contains fibrous material with prominent vascularity. They do not respond to voice therapy and micro laryngeal surgery is needed [25]

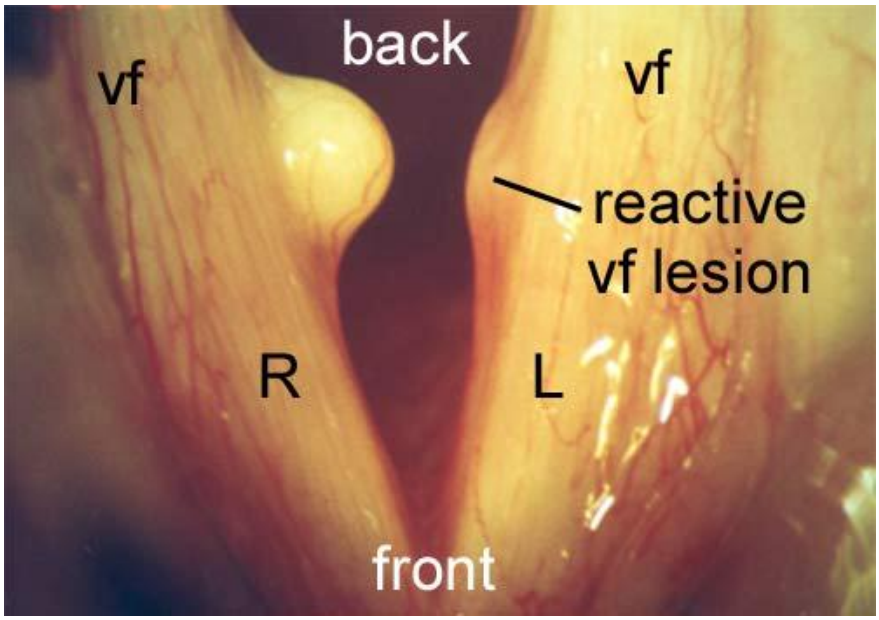

Fig.10 Right vocal cord cyst and left vocal cord reactive lesion

Reactive vocal fold lesions: Reactive vocal fold lesion has to be paired with a contra lateral vocal fold lesion. So it is unilateral. A reactive vocal fold lesion has a cup and saucer appearance, with the contra lateral lesion indenting the reactive vocal fold lesion at the point of maximum contact. The size of the reactive lesion is highly variable with respect to the contra lateral lesion. A reactive lesion will respond to voice therapy [25].

Non specific vocal fold lesions (NSVFL): Vocal fold lesions in which the patient had symptomatic improvement with conservative therapy (non surgical), without resolution of the lesion size. Patients in this group had persistent lesions, but the lesions could not be defined further without a surgical exploration i.e. laryngoscopy and biopsy. The benign nature of these lesions as well as the reduction of the voice symptoms makes surgery avoidable. So a definitive diagnosis of the vocal fold lesion is not reached as the patient improved with non surgical therapy. These patients are described as having non specific vocal fold lesions NSVFL) [25].

The main symptoms on presentation of vocal fold lesions are hoarseness of voice, cough, foreign body sensation and throat pain[33]. The evaluation of the benign lesion is by indirect laryngoscopy (IDL) examination clinically and endoscopically using flexible laryngoscopes or with rigid endoscopes (70 degree or 90 degree). The most accurate investigation is stroboscopy which gives an idea on characteristics of the lesion and about the mucosal wave pattern. Recently for diagnostic purposes, the use of innovative technology such as ultra sonography and virtual laryngoscopy is being explored[34]. The benign vocal fold lesions can be managed with conservative therapy including voice therapy and anti reflux measures. Micro laryngeal surgery and voice therapy is the mainstay of treatment for benign vocal fold lesions. With the introduction of lasers, lesions can be precisely excised. The treatment for benign lesions of the vocal folds consists of a triad of microlaryngeal surgery, voice rest and vocal rehabilitation [31].

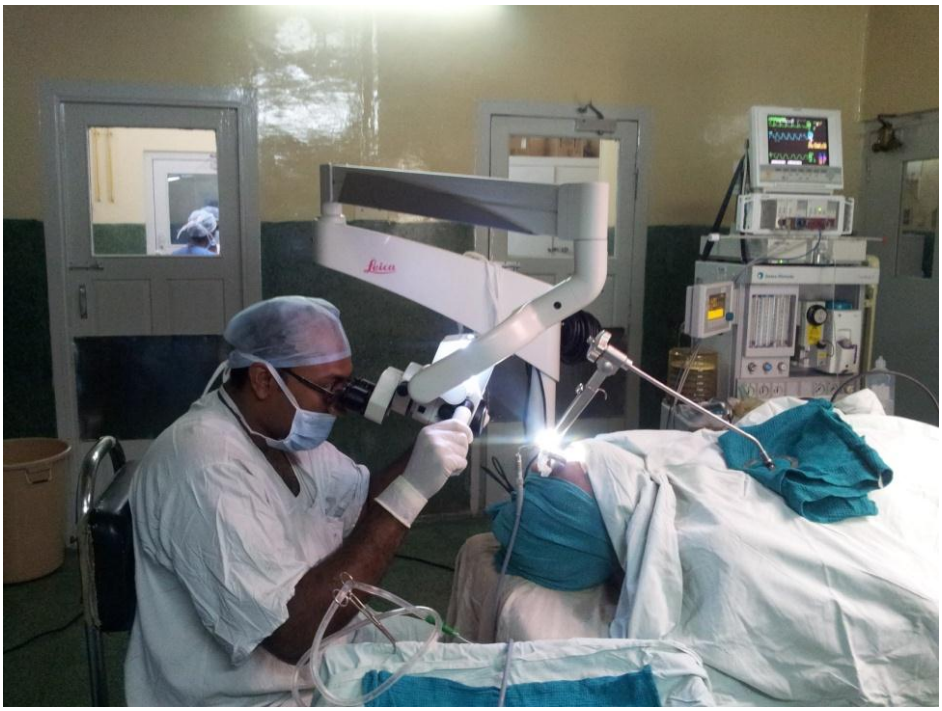

Fig.11 Microlaryngoscopy with chest suspension on the patient.

Pre malignant lesions of the vocal cord

The most accepted definition of neoplasm is the one proposed by Rupert Willis in the 1930s. He described neoplasm as "an abnormal mass of tissue, the growth of which is exceeds and is un coordinated with that of normal tissues, and that persists in the same excessive manner after the cessation of the stimulus which evoked the change"[35]. Transition from normal laryngeal epithelium to squamous cell carcinoma (SCC) is related to progressive accumulation of genetic changes leading to a clonal population of transformed epithelial cells. The whole spectrum of histological changes ranges from squamous hyperplasia to carcinoma in situ (CIS). Even after extensive research into the genetic changes in laryngeal carcinogens, reliable genetic markers

with diagnostic and prognostic value are still lacking. 90% of laryngeal carcinomas are developing from pre malignant lesion[36]. World Health Organisation (WHO) defined pre malignant lesions as "morphological alterations of the mucosa caused by chronic local irritative factors or referable to local expression of generalised illness, presenting a higher probability of degeneration into carcinoma with respect to surrounding mucosa"[37]. The diagnosis of a pre malignant lesion of the larynx must be based on the histological characteristics of the lesion [34]. The various histological classifications are based on the presence of hyperplasia and/or dysplasia. Even though various authors proposed different grading systems, the commonly accepted are mainly three classifications, The WHO classification (2005), Squamous Intra epithelial Neoplasia (SIN) and Ljubljana classifications[38]. Very early lesions show hyper keratosis or para keratosis without cellular atypia or dysplasia. Squamous cell dysplasia is characterised by cellular atypia, loss of normal maturation and stratification short of CIS. In mild dysplasia, the cellular abnormalities are minor and are limited to the basal one third of basal epithelium. Moderate dysplasia shows cellular abnormalities involving up to two third of the epithelial thickness. Severe dysplasia has cellular abnormalities involving more than two third of the epithelial thickness. Carcinoma in situ (CIS) is an intra epithelial neoplasm in which the full thickness of the squamous epithelium shows the cellular features of carcinoma without violation of the basement membrane and stromal invasion[39].

The World Health Organization (WHO) classifies [37] pre malignant laryngeal lesions into six groups as

1. Hyperplasia
2. Keratosis

3. Mild dysplasia
4. Moderate dysplasia
5. Severe dysplasia
6. Carcinoma in situ (CIS)

According to SIN (Squamous Intraepithelial Neoplasia) classification [36], there are only three groups are present

1. SIN 1
2. SIN 2
3. SIN 3

According to Ljubljana Classification, the pre malignant lesions of larynx are divided into four groups [5],

1. Simple Hyperplasia
2. Abnormal Hyperplasia
3. Atypical Hyperplasia
4. Carcinoma in situ

In this classification, the Simple Hyperplasia (SH) is a benign group, Abnormal Hyperplasia (AbH) is a benign group, Atypical Hyperplasia (AtH) is a potentially malignant group and Carcinoma in situ (CIS) is malignant.

The comparison of various grading is as follows:

2005 WHO Classification	Squamous Intraepithelial Neoplasia (SIN)	Ljubljana Classification Squamous Intraepithelial Lesions (SIL)
Squamous cell hyperplasia		Squamous cell (simple) hyperplasia
Mild dysplasia	SIN 1	Basal/parabasal cell hyperplasia*
Moderate dysplasia	SIN 2	Atypical hyperplasia**
Severe dysplasia	SIN 3***	Atypical hyperplasia**
Carcinoma in-situ	SIN 3***	Carcinoma in situ
*Basal/Parabasal cell hyperplasia may histologically resemble mild dysplasia, but the former is conceptually benign lesion and the latter the lower grade of precursor lesions		
** 'Risky epithelium'. The analogy to moderate and severe dysplasia is approximate		
*** The advocates of SIN combine severe dysplasia and carcinoma in-situ		

Table.1 Comparison of various classifications of pre malignant lesions.

Further progression of premalignant lesions of the vocal cord lead to malignancy, mostly squamous cell carcinoma where the tumour is staged as follows according to the TNM classification.

T_1 - Tumour limited to vocal cord(s) (may involve anterior and posterior commissure) with normal mobility.

T_1a – Tumour limited to one vocal cord.

T_1b – Tumour involves both vocal cords

T_2 – Tumour involves supra glottis and / or subglottis and /or with impaired vocal cord mobility

T_3 – Tumour limited to larynx with vocal cord fixation and/or invades paraglottic space and / or minor thyroid cartilage erosion.

T_4a – Tumour invades through thyroid cartilage and / or invades tissue beyond the larynx (eg: trachea, soft tissues of neck including deep extrinsic muscles of the tongue, strap muscles, thyroid or oesophagus)

T_4b – Tumour invades pre vertebral space, encases carotid artery or invades mediastinal structures.

Simple and abnormal hyperplasia is considered benign forms with 0.7% and 1% risk of malignant transformation respectively [5]. Malignant transformation of mild dysplasia is reported

as 11%[40]. Atypical and severe dysplasia are associated with highest risk of cancer ranging from 19% to 28%[41, 42]. 30% of patients with dysplastic lesions that progressed to invasive cancer eventually underwent total laryngectomy[38]. Almost all diffuse lesions showing CIS developed invasive cancer[43].

Malignant transformation is a multi step process that takes many years. Blackwell and colleagues observed a 3.9year interval between initial biopsy to progression to invasive cancer [40]. In a series of more than 1000 keratosis larynx, the mean time interval to malignant transformation was 3.1 years[44]. The latency was longer for earlier lesions, and 7% of lesions transformed into invasive carcinoma more than 10 years after the initial biopsy. Lesions showing mild dysplasia and even those without dysplasia may progress into invasive cancer. A long term follow up of all pre malignant lesions is warranted as they may develop into carcinoma even after many years[45].

Fig.12 Bilateral vocal cord dysplasia

The laryngeal pre malignant lesions have no specific macroscopic appearance. They are clinically referred as[40]

1. Chronic laryngitis.
2. Keratosis
3. Leukoplakia
4. Erythroplakia
5. Hyperplastic-dysplastic lesions.

The surface morphology and keratin layer formation of these lesions has no specific meaning, nor any significant relationship with their malignant potential[46]. The histopathological diagnosis guides a clinician for the treatment of patients with benign, potentially malignant or actually malignant lesions.

Fig.13 Left vocal cord keratosis

Management of pre malignant lesions: Conservative measures can be tried for one month in the absence of a) worsening of vocal symptoms, b) an enlarging lesion c) clinical signs of invasive carcinoma. Conservative measures include proper hydration, elimination of vocal abuse; reduce the risk factors especially to stop smoking and alcohol intake. All patients with laryngopharyngeal reflux should be treated with proton pump inhibitors [5].

Chemoprevention with retinoid, selenium and other agents are still a matter of debate. However clinical response to retinol palmitate for laryngeal hyperplasia with an induction dose of 3,00,000 IU followed by a maintenance dose of 15,00,000 IU was assessed by Issing and colleagues[47]. There was a complete response in 75% of patients and a partial response in the remaining. None of these lesions progressed to cancer. One principal drawback to using retinoids is that the lesions tend to recur when the treatment is discontinued.

The management decision is mainly depending on whether there are single or multiple lesions or widespread disease [5]. Single and multiple lesions should be completely excised to all visible margins, if possible. In case of wide spread disease, histopathological mapping of the lesion with multiple biopsies should be performed initially followed by a staged resection if feasible. Other factors which are important in deciding the management are patient's general condition, fitness for surgery, age, co morbidity and presence of other risk factors [5]. The treatment of a premalignant lesion should aim to eradicate the lesion while preserving the voice quality and laryngeal function. Accurate histopathological diagnosis is critical for proper decision making. Hyperplastic and dysplastic lesions are excised with microlaryngoscopic techniques to remove the visible lesions. Close follow up is required due to risk of recurrence of the lesion and chance of malignant transformation. Carcinoma in situ may be treated with either

surgery or radiotherapy (RT). Surgical management is microlaryngoscopic excision. The carbon dioxide (CO2) laser is useful for precise excision, although thermal injury to underlying lamina propria may occur. Vocal cord stripping is the removal of the mucosa of the vocal fold including the lesion from the vocal process up to the anterior commissure. Surgical treatment is preferred for focal lesion in patients who are reliable and will attend routine follow up. Radiation therapy is also an effective treatment for Carcinoma in situ (CIS). A recent study of Carcinoma in Situ (CIS) treated with RT shows that the local control rate was 93.5%[38]. Radiation therapy is particularly useful for patients with multiple recurrences following surgical excision, for diffuse lesions, for patients who can't attend regular follow up and for patients unfit for general anaesthesia. Voice quality is well preserved following radiation therapy[48, 49].

In recent years, in office management of pre malignant lesions has become more common. This includes the treatment of pre malignant lesions with fibre based laser systems such as the Pulsed Dye Laser (PDL) and the pulsed KTP laser and by Photo Dynamic Therapy (PDT).

The 585nm PDL and 532nm pulsed KTP lasers are the two most widely used lasers for the in office treatment of laryngeal lesions including leukoplakia, keratosis and dysplasia. In a study by Franco and colleagues, PDL used for the treatment of dysplasia in the operating room under general anaesthesia. In most cases the treated lesions were then excised and sent for histopathological examination. The treatment was effective: 81% of patients had greater than 70 % of regression of their lesion[50]. The same authors demonstrated the efficacy of PDL delivered via a flexible endoscope in the office for the treatment of dysplasia[51]. Zeitels et al have adopted the pulsed KTP laser for in office use, citing smaller fibre diameter, greater reliability, improved

intra lesional energy absorption, and improved haemostasis as advantages of the pulsed KTP system over PDL[52]. The energy from these lasers is preferentially absorbed by oxyhaemoglobin, with the absorption of energy from the pulsed KTP being superior to that of PDL, causing photo angiolysis of the sublesional blood vessels[50]. Preferential destruction of intraepithelial desmosome junctions and separation of the treated epithelial cells from the basement membrane have also been observed microscopically[53]. Apart from the obvious advantages of in-office procedures (avoidance of general anaesthesia, lower cost, improved efficiency, and patient preference), these lasers have minimal effect on the surrounding tissue and the scarring is uncommon. The bilateral simultaneous treatment of lesions in and around the anterior commissure is possible, with minimal risk of web formation[54]. One of the major criticisms of in-office procedures for the treatment of laryngeal lesions, especially dysplasia, is that there is no specimen obtained for pathologic examination, and therefore a definitive assessment of the tumour extent and margin status is not possible. In the reports published to date, all patients have been biopsied before the commencement of treatment [50, 52.] A biopsy may be obtained in the operating room and initial treatment with the laser given at this time, or it may be taken in the office via the endoscope. Potential complications of in-office treatment include poor exposure of the lesion or poor patient tolerance of the procedure; however, these issues only arise in a small minority of cases [52]. Further studies with long-term follow-up are awaited to ensure that this novel technology has similar outcomes for patients with dysplastic laryngeal lesions.

Photodynamic therapy uses nontoxic chemicals that are taken up preferentially by dysplastic or malignant cells. These photo reactive chemicals are activated by light of a specific frequency range unique to the compound used. The light activation of tissues at the target site in the presence of oxygen results in cell death that is confined to the cells that have selectively

accumulated the chemical[55]. Several agents have been used in the head and neck cancers including 5-ALA, Photofrin, haematoporphyrin derivative, and Foscan. In the United States several photosenstitisers have been approved for use by the Food and Drug Administration, but treatment of head and neck malignancies is an off-label use at the present time. In the European Union, Foscan has been approved for treatment of early head and neck cancer. At present, data are available for approximately 1500 subjects treated with PDT for head and neck SCC [53]. Biel reported on 115 patients with Tis to T2 larynx SCC treated with Photofrin PDT. With a mean follow-up of 91 months, the 5-year cure was 91%. All recurrences were salvaged successfully [53]. Photodynamic therapy has several advantages: it is performed in an outpatient setting and is repeatable. The effects on voice quality are minimal, and scarring of the vocal folds has not been observed [53]. Careful light-avoiding precautions are required because light sensitivity lasts up to 4 weeks. There is no pathologic specimen, and care must be taken to accurately assess tumour depth. PDT may have a particularly useful role for diffuse dysplasia or CIS. Schweitzer reported 80% local control with PDT in 10 cases of diffuse laryngeal CIS previously treated with RT[56]. Published reports show promise for the use of Photo Dynamic Therapy (PDT), but currently it is used only in selected centres and is not widely available.

Screening for Laryngeal carcinoma

Attempts to screen the laryngeal cancer have met with little success as the populations who would benefit most from screening are those least likely to seek it, especially the elderly male population with low socio economic status. The standard of screening for laryngeal carcinoma is by flexible laryngoscopy by an experienced surgeon or the review of the images or videos of the laryngoscopy by an expert. This makes the screening not cost effective and time

consuming. An acoustic screening method to find out the possibility of laryngeal cancer has been investigated[57]. Three acoustic parameters named pertuberations in pitch period, peak amplitude sequences and vocal noise of a sustained vowel, "e" were measured. The combined use of these parameters enabled to build an acoustic screening system for laryngeal cancer [55]. But the routine use of this system is not known. In a study by Fischinger J, and Mlacak B[58] in Slovania, the authors concluded that screening does not always result in the hoped for outcome. It also requires much time and effort. They even question the justification of yearly examinations of the populations at risk, even though some authors consider this to be feasible. More important than screening is public health education which is primarily the responsibility of general practitioners as well as the laryngologists [56].

Studies on techniques to diagnose premalignant and malignant lesions of vocal cords

The visual appearance of a premalignant laryngeal lesion does not predict its histologic nature. Pre operative investigations like videostroboscopy also do not reliably differentiate premalignant from malignant lesions[59]. Biopsy is the gold standard for diagnosis and adequate sampling of the suspected area is important for the management. Various adjunctive techniques have been developed to improve the clinician's ability to characterise these lesions, to guide biopsies and to aid in resection of the vocal cord lesions intra operatively using microlaryngoscopic techniques or by laser resection.

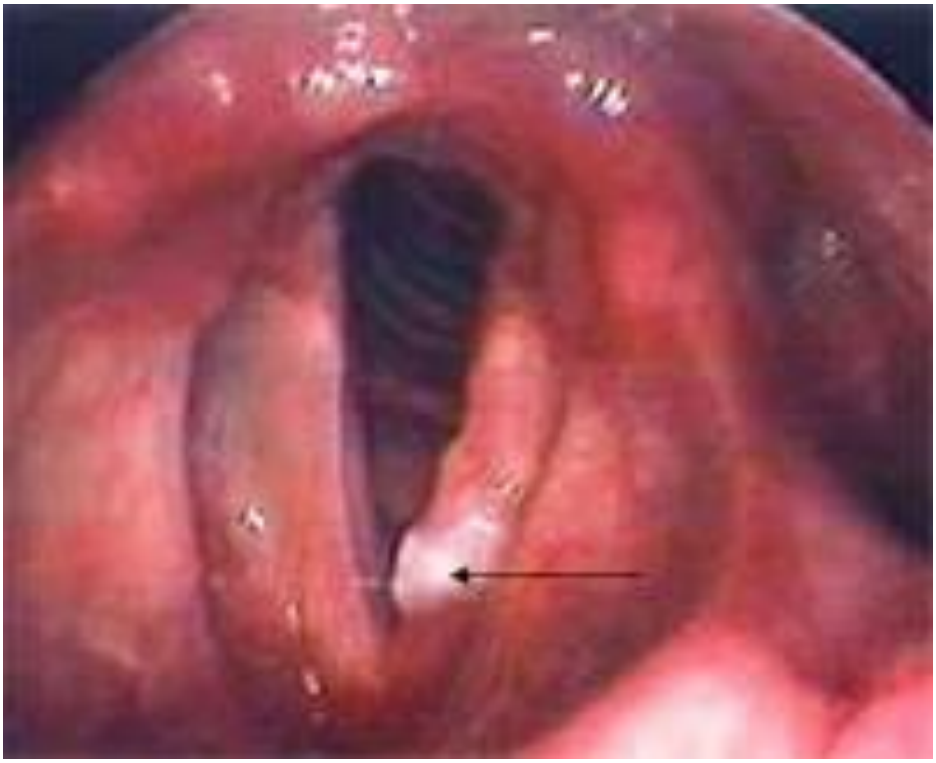

Fig.14 Malignant lesion left vocal cord.

The various methods which are used for detection of pre malignant and malignant lesions are

1. Vital dyes like toluidine blue and methylene blue.
2. Contact endoscopy
3. Autofluorescence endoscopy
4. Induced autofluorescence using 5-aminolevulinic acid (5-ALA)
5. Compact endoscopy
6. Optical coherence tomography (OCT)

Vital dyes are chemicals which can be used safely in live tissues. The use of vital dyes like toluidine blue and methylene blue as an adjunctive tool for the evaluation of pre malignant lesions have been investigated[60, 61]. Lundgren and colleagues used toluidine blue to detect dysplasia or malignant changes and noted 91% sensitivity, but only 52% specificity [59]. Even though Lugol's iodine is a vital dye and it has been using in the lesions of the oral cavity, oropharynx and oesophagus, no studies have investigated its use in vocal cord lesions.

Contact endoscopy provides magnification of 50 to 60 times and, with methylene blue staining, provides histologic information and assessment of vascular patterns[62]. However, the subsurface visualisation is limited only to 150 to 200 microns and is inadequate for characterizing thicker lesions. Widespread use of this technique has been limited by the need for extensive experience to interpret the findings and for the special equipment.

Human tissue has many compounds that fluoresce when exposed to blue light. The difference in fluorescence of abnormal tissues has been exploited as a diagnostic tool for laryngeal malignancy. Limitations include the need for adequate experience in the technique, false-positive and false-negative examinations in scarring, hyperkeratotic lesions, and inflammation[63].

Induced autofluorescence using 5-aminolevulinic acid (5-ALA) also shows potential diagnostic utility. 5-ALA selectively induces accumulation of protoporphyrin IX fluorescence in tumours. Using a nebulizer, it is topically applied 1 to 2 hours before the examination and a light source emitting short wavelength visible light (375 to 440 nm) is used to induce fluorescence. Protoporphyrin IX fluorescence appears red during imaging. In a study by Mehlmann et al, a

series of 16 patients with suspected or proven laryngeal malignancy subjected to 5-ALA–induced fluorescence endoscopy demonstrated a sensitivity of 95% and a specificity of 80%[64].

A prospective evaluation of autofluorescence and 5-ALA–induced fluorescence in 56 patients demonstrated that the two techniques have similar sensitivities (94% and 97%) and specificities (82% and 64%) to distinguish hyperplasia or mild dysplasia from moderate dysplasia, severe dysplasia, CIS, and invasive SCC[65]. Autofluorescence was unreliable when scarring was present, whereas 5-ALA–induced fluorescence resulted in false-positive findings in inflamed lesions.

The combined use of autofluorescence and contact endoscopy ("compact endoscopy") has also been investigated. A study by Arens and colleagues of 83 patients with hyperkeratotic, dysplastic, and invasive laryngeal lesions found correlation with histology in 88% of cases [63]. Inflammation and scarring resulted in overestimation of disease, whereas underestimation of disease was seen in hyperkeratotic lesions.

Current non-invasive diagnostic modalities do not reliably distinguish premalignant lesions from invasive SCC. Optical and microscopic imaging is limited by the inability to see below the first few layers of epithelial cells to evaluate the submucosal architecture. Infrared light has increased tissue penetrance and can provide diagnostic information about subsurface tissues. Optical coherence tomography (OCT) is a new diagnostic modality under investigation to examine the epithelial and subepithelial architecture that uses near-infrared light waves to produce cross-sectional images of tissue in vivo, with a resolution nearing that of histology[66, 67]. In the larynx, OCT clearly identifies basement membrane violation from laryngeal cancer and is able to identify transition zones at the margins of the cancer[68]. OCT has been used to assess the

larynx intraoperatively to guide the extent of transoral laser resections for laryngeal cancer to ensure that excision is complete[69]. The primary disadvantage of OCT is the limited depth of penetration (<2 mm), which limits its utility in bulky lesions. However, OCT has potential as a useful tool in the management of laryngeal cancer: for the diagnosis of smaller epithelial lesions, for guiding surgical biopsies and resections, and for monitoring larynges post treatment.

Lugol's iodine: Basic facts and studies on effects of Lugol's iodine in oral cavity, oropharynx and oesophagus.

The vital dyes are an auxiliary technique used in vivo to evidence suspicious lesions and to better define the existing lesion's margin and extension [6]. These vital dyes are capable of penetrating living cells and binding to specific biological structures.

Lugol's iodine, also known as Lugol's solution first made in 1829, named after the French physician JGA Lugol (1786-1851), has a high affinity for glycogen in non keratinized squamous epithelium[70].

Lugol's iodine is a solution of elemental iodine and potassium iodide in water. Original Lugol's solution contain 5gms of iodine and 10gms of potassium iodide mixed with 85ml of distilled water, with a total iodine content of 150mg/ml [6]. The first use of iodine staining to detect mucosal abnormalities was described by Schiller, the Austrian – American gynaecologist and pathologist in 1933. He used this technique to highlight squamous lesions in the cervix[71], hence the name Schiller's test, came for vital dye with Lugol's iodine.

Fig.15 Lugol's iodine

The principle of iodine staining is that iodine reacts with glycogen in the cytoplasm, and this reaction is known as **Iodine - starch reaction**, is visualized by a colour change. Tissue glycogen content is always related to the degree of keratinisation. Glycogen content is inversely proportional to the degree of keratinisation [6]. Iodine is glycophilic and the application of Lugol's iodine solution results in uptake of iodine in normal glycogen containing epithelium and stains the mucosal surface to Mahogany brown or black[72]. In para keratinised stratified squamous epithelium, the cells in the intermediate and superficial layer contains glycogen in their cytoplasm. In contrast dysplastic or invasive cancer cells contain little glycogen and these areas do not take up iodine and remained as unstained lesions [70]. Various concentrations of Lugol's

iodine solution were used in different studies: 1.25%, 1.5%, 2%, 3%, 5% and 10% (65=6). In a study by Sreedharan et al [73], the authors recommend the use of 1.5% of Lugol's iodine in the chromoendoscopic studies in oesophagus.

Fig.16 Application of Lugol's iodine in the evaluation of tongue lesions. The lesion shows unstained pattern

In a study by McMahon J et al [70], they used Lugol's iodine in the resection of oral and oropharyngeal squamous cell carcinoma. Visualisation of intra epithelial neoplasia with Lugol's iodine is the method chosen for the margin of resection. They suggest that Lugol's iodine is a simple, inexpensive, and apparently effective means of reducing the likelihood of unsatisfactory surgical margins in the resection of oral and oropharyngeal squamous cell carcinoma.

In a study by Maeda K et al[74], colorimetric analysis of unstained lesions surrounding oral squamous cell carcinomas and oral potentially malignant disorders using iodine were studied. They noted that after using 3% Lugol's iodine, the colour difference allow easier detection of unstained lesions with a clear border, which helps in resection of the lesion in oral cavity. They conclude that the colour charts prepared on the basis of measures of lightness and hue reproduced the macroscopic colour differences in unstained lesions(USL), suggesting that it may become possible to diagnose unstained lesions (USL) histologically on the basis of the measured colour values and use of colour charts to help determine the resection area in surgery.

Massimo and colleagues [6], reviewed studies on the use of Lugol's iodine in oral cancer diagnosis. They found out that Lugol's iodine has a sensitivity of 0.875 and specificity of 0.842 in detecting oral squamous cell carcinoma. It has less sensitivity in identifying oral malignant and dysplastic disease but it was of greater specificity. The authors conclude that, all the studies analysed found the Lugol's solution to be effective, cheap and easy to use and they emphasized its importance in clinical practice.

Yajima et al [75], quantified the telomerase activity of unstained areas (after application of Lugol's iodine) surrounding oral squamous cell carcinoma. In all cases the unstained areas showed a statistically significant increase in telomerase activity, thus confirming the role of Lugol's solution in detecting the margin of oral squamous cell carcinoma.

In a study by Stanford and colleagues[76], describes the use of Lugol's iodine in detecting high grade squamous dysplasia and invasive carcinoma, in oesophagus during oesophagoscopy. They found out mucosal iodine staining is a very sensitive technique for detecting the precursor and early invasive lesions of squamous oesophageal carcinoma.

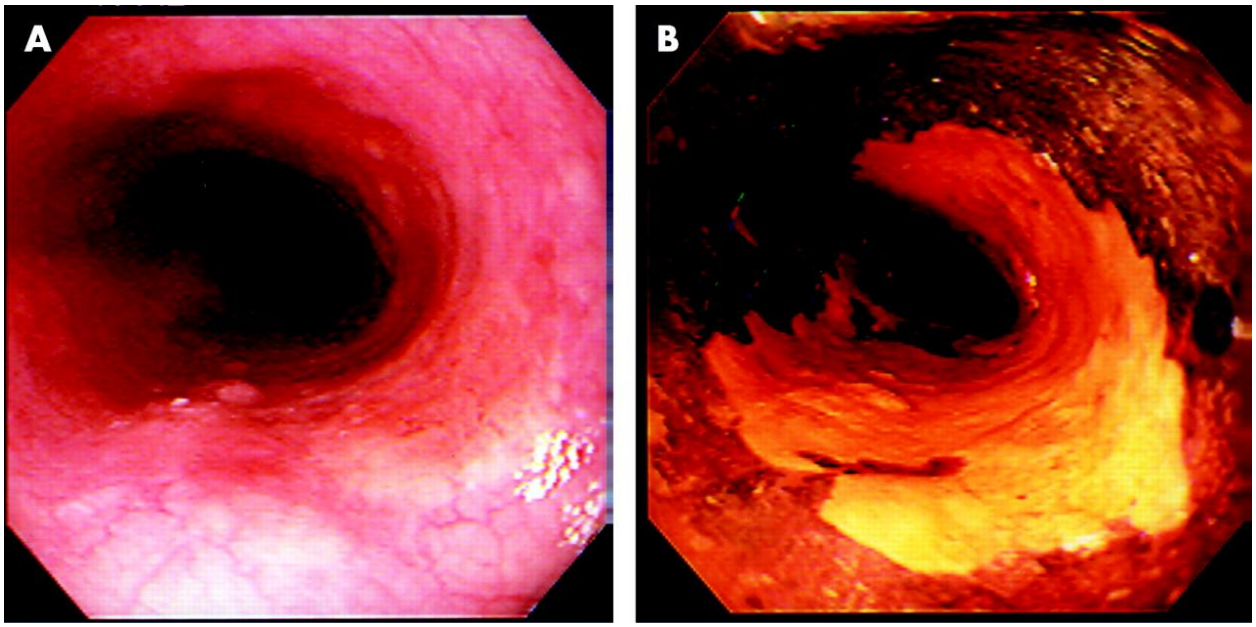

Fig.17. Application of Lugol's iodine in oesophagoscopy to detect pre malignant and malignant lesions (as evidenced by unstained areas)

They recommend the use of Lugol's iodine for optimal visualisation of oesophageal squamous mucosal abnormalities during endoscopy (oesophagoscopy). In that study after staining with Lugol's iodine, the sensitivity of USL (Un Stained Lesions) for identifying high grade dysplasia or carcinoma was 96% and the specificity was 63%. They also noted that after staining with Lugol's iodine the lesions were larger or more clearly defined. They concluded that the use of Lugol's iodine during oesophagoscopy is safe, inexpensive, simple and rapid to perform, easy to interpret and highly sensitive for all clinically important squamous neoplasia. It is essential for detecting many squamous dysplasias and for detecting the full extent of the

lesion. It should be used as a routine procedure in high risk populations and whenever an unexplained squamous mucosal abnormality is observed in low risk individuals [74.]

In effect Lugol's Iodine is a routinely used vital stain in UADT malignancies screenings, it is safe, inexpensive, simple and rapid to perform, easy to interpret and highly sensitive for all clinically important squamous neoplasia. According to our search and understanding, the use of this method is not studied in glottic malignancies, hence this study.

MATERIALS AND METHODS

The purpose of the study is to identify the staining properties of the Lugol's iodine in various vocal cord lesions and investigate the effectiveness of Lugol's iodine in detecting various pre malignant and malignant vocal cord lesions.

Type of study: Observational study

Period of study: 1 year

Settings: This study was performed in the ENT outpatient department and Operating room at Christian Medical College, Vellore, between December, 2011 and November, 2012. All patients aged 18 years and above with hoarseness of voice for more than 4 weeks, and were scheduled to undergo microlaryngoscopic evaluation / surgery, were included in the study.

Inclusion criteria:

1. Patients with hoarseness of voice for more than 4 weeks.
2. Age above 18 years.
3. A written informed consent
4. No history of allergy to iodine.

Exclusion criteria:

1. Patients who had undergone any surgery of the larynx or received any chemotherapy or radiotherapy for head and neck cancer.

2. Distant metastasis.
3. Any history of allergy to iodine or any contrast material.

Sample size calculation:

Taking into consideration that Lugol's iodine has been used in the detection of oral squamous cell carcinoma, where it has been shown to have 85% sensitivity [6] and applying a precision of 10% and a confidence interval of 80%, a sample size of 88 was calculated.

The sample size was calculated using the formula,

$n = 4PQ/d2$,

where,

n = sample size

P = sensitivity

$Q = 100 – P$

d = precision.

Material: Lugol's Iodine solution, 1.5% of concentration is used in the study. It is prepared by mixing 1.5gm of Iodine and 3gm of Potassium Iodide in 100ml water. The cost of Lugol's iodine is Rs. 1.10 per ml, which is cheaper than any dye used in the various studies including Toluidine blue. 1 ml of the dye is sufficient for use in single patient.

Methods:

Study population recruitment: Consecutive patients presenting in the ENT outpatient department with hoarseness of voice for more than 4 weeks and fulfilling inclusion and exclusion criteria, and were planned for microlaryngoscopic examination / surgery under general anaesthesia, were recruited after obtaining a valid informed consent. Pre-operative evaluation included clinical examination and Nasopharyngolaryngoscopy (NPL scopy) using flexible laryngoscope (Karl Storz, Germany). The findings were noted and video recording of the lesion done using 'IMIMO' recording software (Avanttec Medical Systems Pvt Ltd). The findings were noted in the proforma (Appendix).

Procedure: Under general anesthesia patients were examined by direct laryngoscopy and the suspected vocal cord lesion was visualised under microscope (Karl Zeiss) with a 400mm objective lens and 6X magnification. Findings noted were recorded. Features of suspected lesion were observed and their characteristic features like polyp / nodule / cyst / keratosis/ growth were recorded. Also the presence of the lesion on unilateral or bilateral cords, colour of the lesion, extent of the lesion, number of lesions (single or multiple etc) was noted.

Applying the dye on the vocal cords: The saliva and mucous over the vocal cords are suctioned out and the remaining film of mucus is removed by wiping the surface of the entire vocal cord including the suspected area with dry cotton ball 3 times. After confirming the entire surface is free of saliva or mucous, a cotton ball dipped in 1.5% Lugol's iodine is applied over bilateral vocal cords in sweeping motion. Care is taken to avoid spillage in to the tracheo-bronchial tree. The dye is allowed to be absorbed for up to 1 minute, after which the staining pattern is observed for upto 3 minutes. Based on staining patterns the lesions are noted as "stained" or "unstained".

Stained - means Brown or Mahogany brown.

Unstained – means remains white/pink/ takes the stain initially and turns to previous colour.

The whole procedure is video recorded using the software Pinnacle Studio. (Pinnacle studio is a non linear video editing software application manufactured by Pinnacle System, a division of Avid Technology). The lesion on the vocal cord is excised using microlaryngoscopic techniques and specimen sent for histopathological examination. The description of the lesion and the staining property are noted in the proforma.

Considering histopathological (HPE) report as gold standard, the study also compares the HPE report with the staining property. Also the study reports the staining property of Lugol's iodine in various vocal cord lesions.

Statistical analysis:

The data of all the patients collected systematically with the software EPIDATA version 3.1.All statistical analysis were performed using statistical software STATA (version 10.0), STATA corporation, Texas, USA. The sensitivity, specificity, positive and negative predictive value calculated using this software.

RESULTS

A total of 89 patients participated in the study, of which 14 patients were having bilateral vocal cord disease. Hence a total of 103 samples were sent for histopathological examination (HPE), and correlated with the staining pattern. The comparison was intended to correlate the unstained pattern of the suspected lesion indicating the presence of a pre malignant or a malignant lesion.

Following are the various parameters that were observed:

1. **Sex ratio of the study population**.

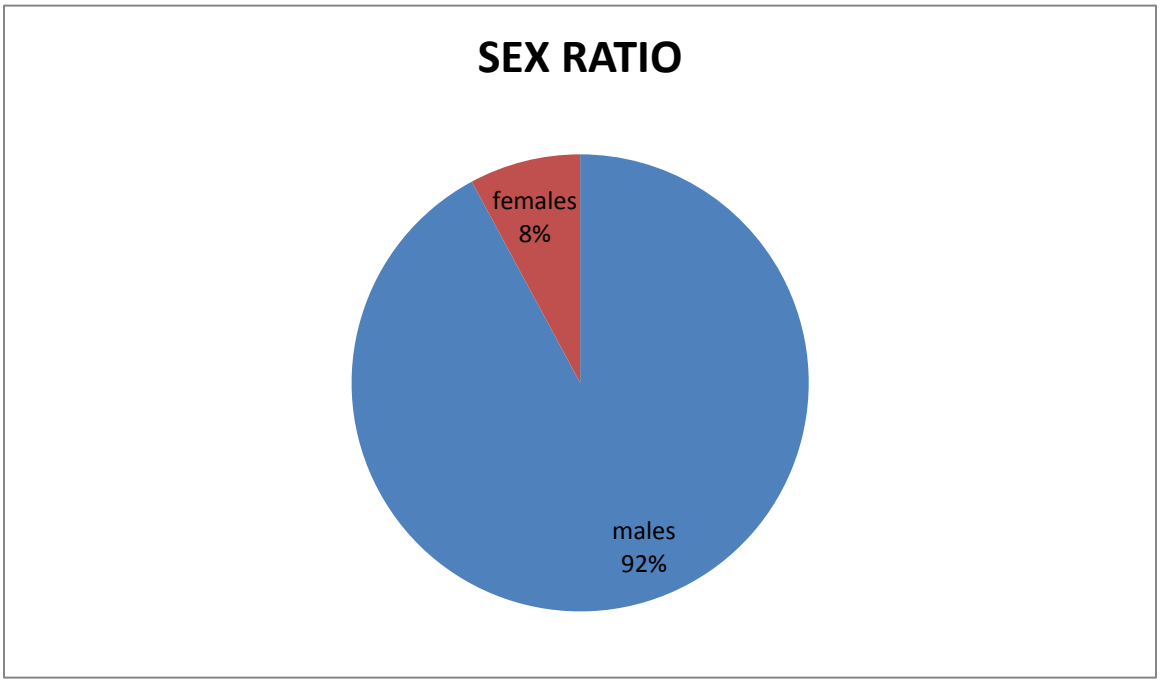

Fig.18 Sex ratio of the patients.

Of the total 89 patients, 92% (82 patients) are males and 8% (7 patients) are females. There is an evident male preponderance for vocal cord disorders.

2. Age distribution pattern

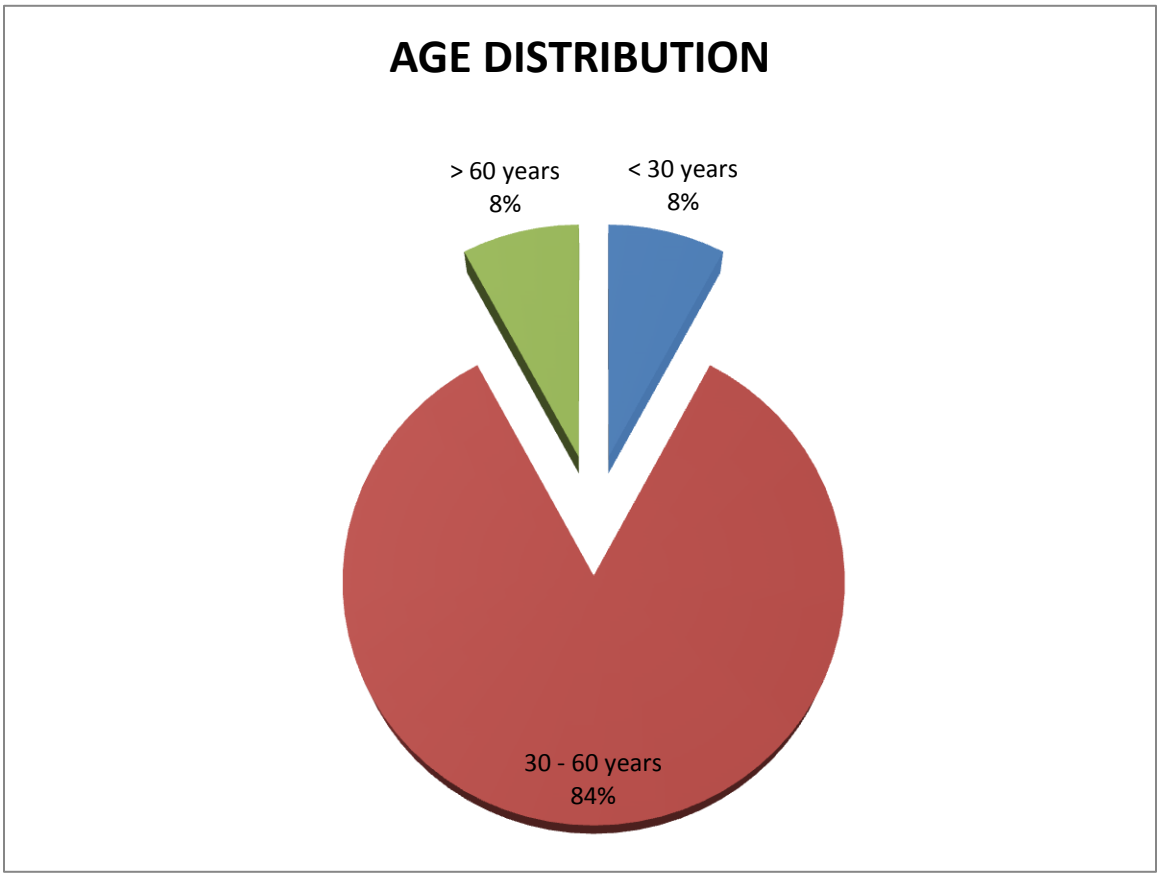

Fig.19 Age distribution of the patients.

The age range was 22 years to 82 years with a mean age of 52 years. Of the total 89 patients, 8% (7 patients) are less than 30 years of age, 84% (75 patients) of patients are between 30 to 60 years, and 8% (7 patients) are more than 60 years of age. This shows that the vocal cord

lesions are present more in the middle aged population than in the younger and elderly population.

3. Duration of hoarseness of voice in study population

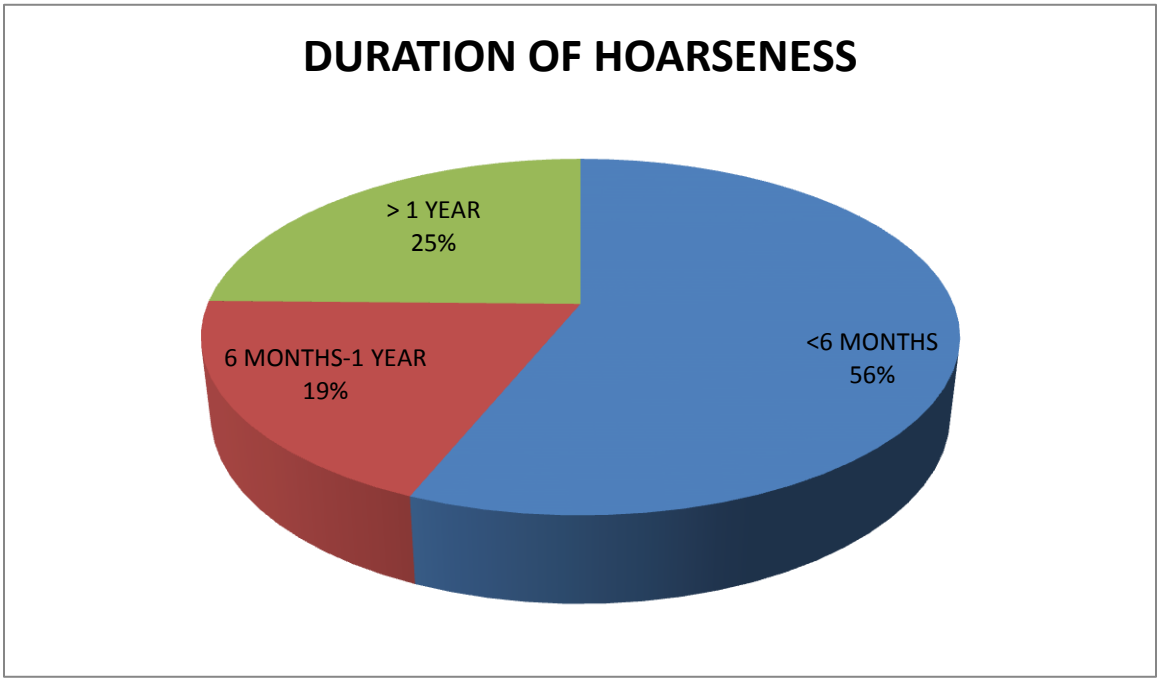

Fig.20 Duration of the hoarseness of voice

In the present study, 56% (50 patients) of patients presented with hoarseness of voice less than 6 months of duration, 19% (17 patients) of patients presented with hoarseness of voice more than 6 months but less than 1 year, and 25% (22 patients) presented with hoarseness more than 1 year duration.

4. **Distribution of vocal cord lesions.**

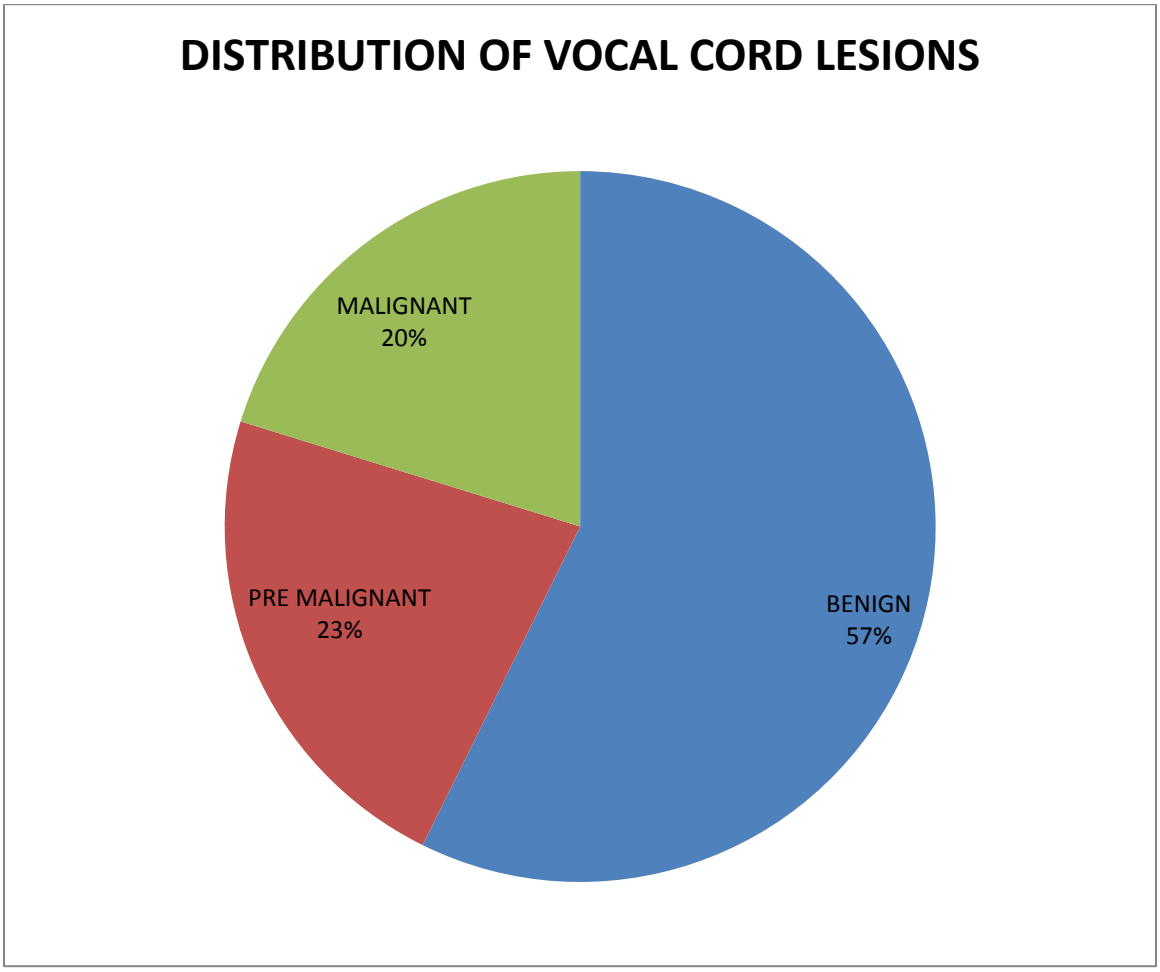

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Fig.21 Distribution of the vocal cord lesions.

A total of 89 patients participated in the study. Of all the patients who presented with hoarseness of voice, 57% of patients (51 patients) were diagnosed to have benign lesions, 23% of patients (20 patients) had pre malignant lesions and 20% of patients (18 patients) had carcinoma.

This study shows that more than half of the patients who presented with hoarseness were having benign lesions.

5. **Sex distribution of various vocal cord lesions**.

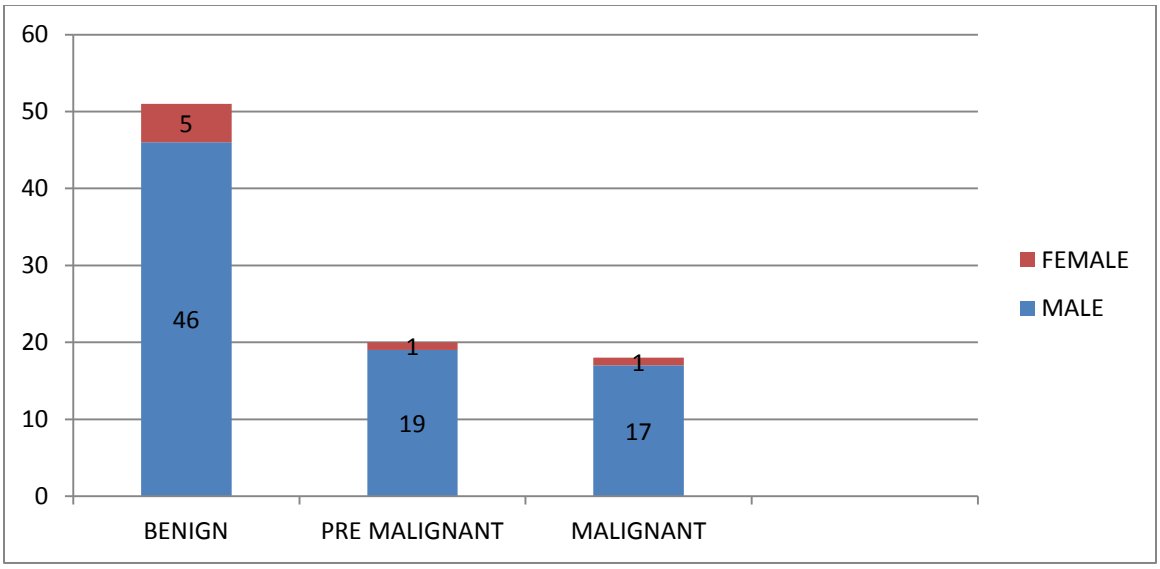


Fig.22 Sex distribution of various vocal cord lesions

Of the total 89 patients 51 patients were diagnosed to have benign vocal cord lesions, of which 46 were male and 5 were females. In the pre malignant group, of the total 20 individuals, 19 were male and only 1 was female. Of the patients who were diagnosed to have malignant lesion, of the total 18 patients, 17 were males and only 1 individual was female. This indicates

an overall male preponderance in vocal cord lesions and particularly in pre malignant and malignant lesions.

6. Distribution of various pre malignant lesions.

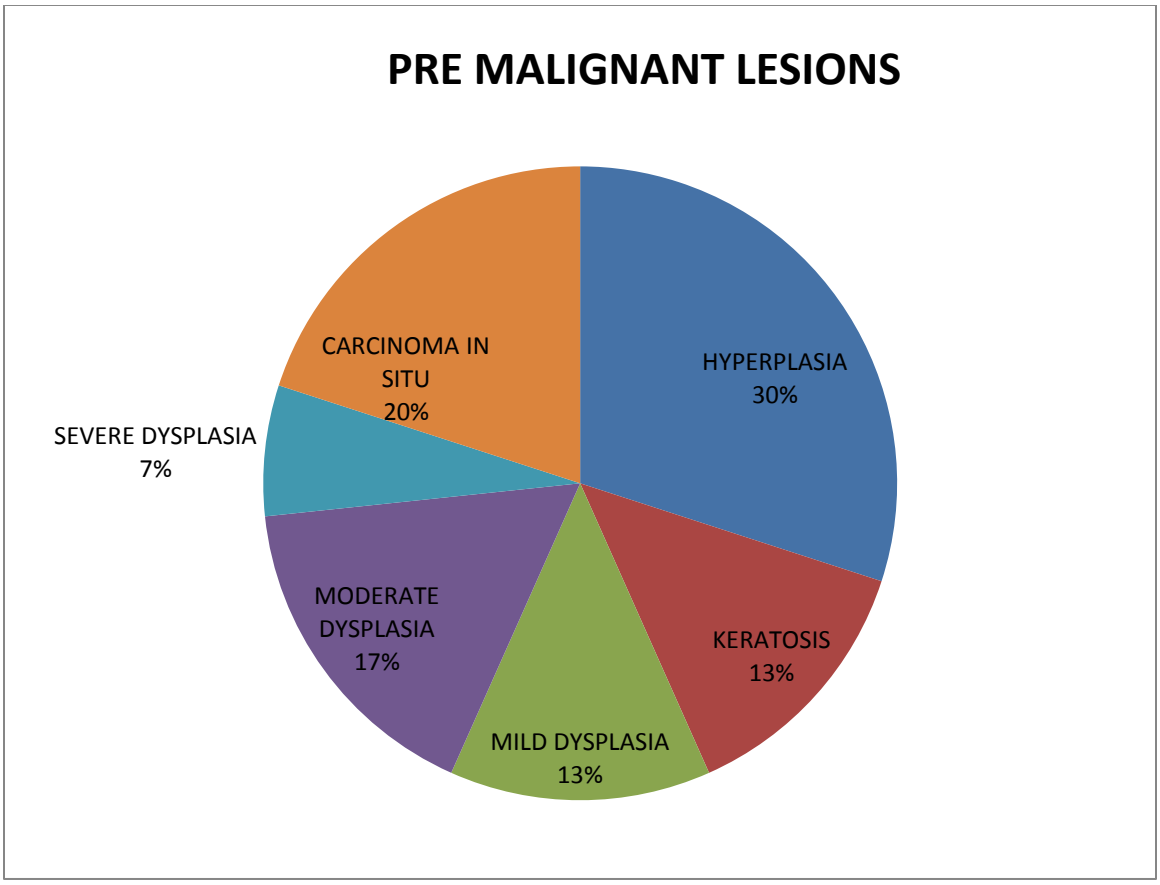


Fig.23 Percentage of various pre malignant lesions.

Of the total 30 pre malignant lesions, 30% is hyperplasia (n=9), 13% is keratosis (n=4), 13% mild dysplasia (n=4), 17% moderate dysplasia (n=5), 7% severe dysplasia (n=2), and 20%

carcinoma in situ (n=6). This shows that hyperplasia is the most reported pre malignant lesion in vocal cords, followed by carcinoma in situ.

7. Laterality of the lesion

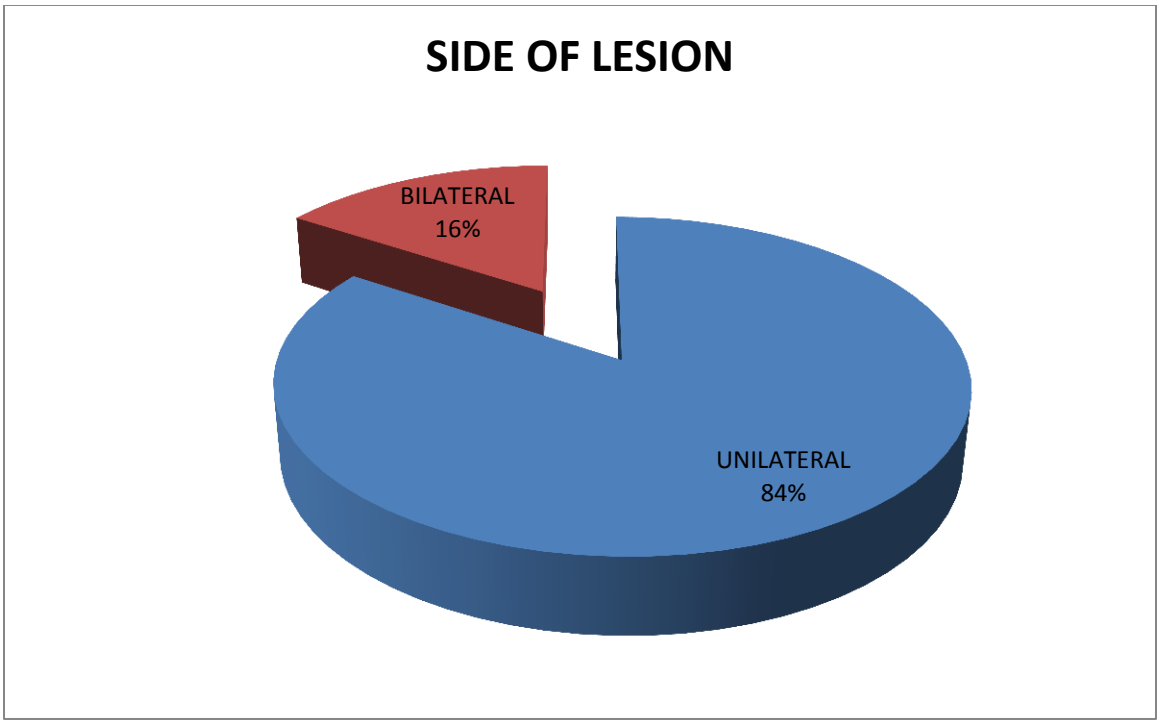


Fig.24 Laterality of the lesion.

Of the total 89 patients, 75 have unilateral lesions and 14 have bilateral lesions. Almost 1 in every 5 cases had a bilateral lesion.

8. Presenting complaints of vocal cord lesions

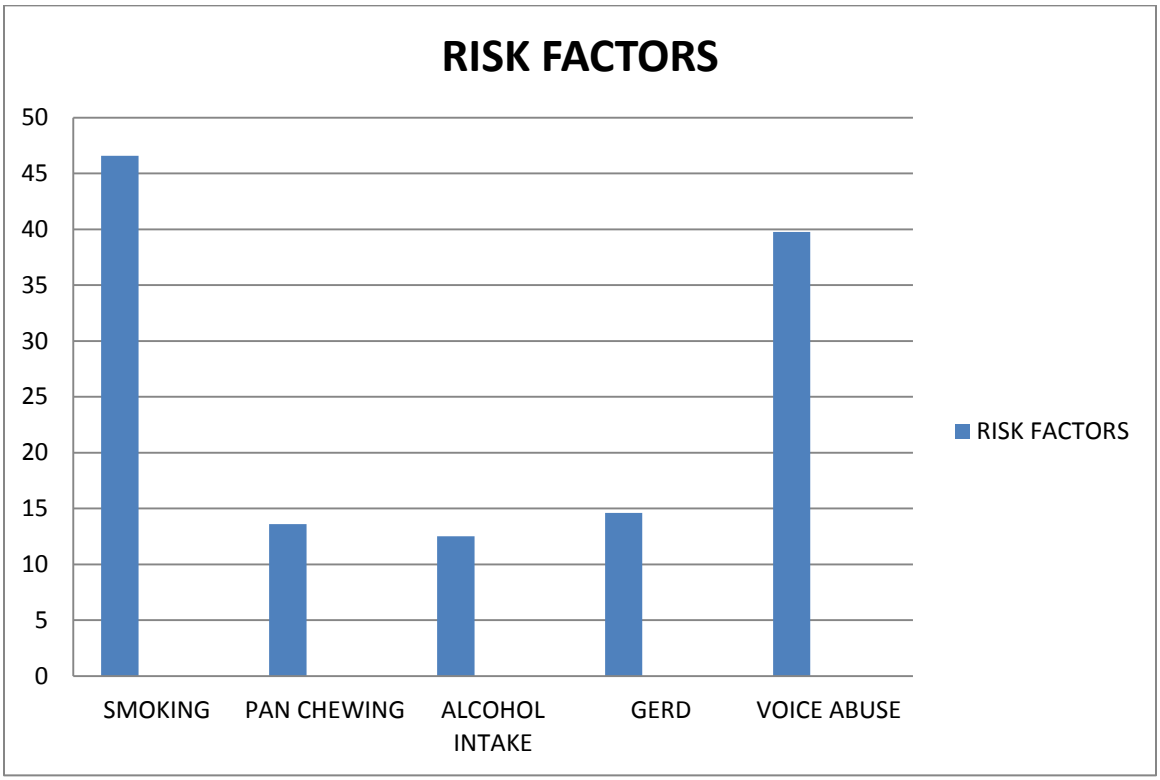
Presenting complaints	Number of patients
Hoarseness of voice	89
Globus pharyngeus	9
Dysphagia	0
Noise breathing	4
Neck swelling	0
Bilateral ear pain	1
Haemoptysis	1
Odynophagia	0

.

Table.2 Presenting complaints of vocal cord lesions

Each of the 89 patients in the study presented with hoarseness of voice of more than 4 weeks of duration (n=89). Other associated complaints were Globus pharyngeus (n=9), noisy breathing (n=4), dysphagia (n=0), odynophagia (n=0), neck swelling (n=0), bilateral ear pain (n=1), haemoptysis (n=1). Globus pharyngeus is the second commonest symptom.

9. Risk factors of various vocal cord lesions



. **Fig.25 Risk factors for various vocal cord lesions**

The various risk factors which are analysed in our study are smoking, pan chewing, alcohol intake, gastro esophageal reflux disease (GERD), and voice abuse. Smoking was reported in 46.59% of patients (41 patients) and it is the most common risk factor in vocal cord lesions. Pan chewing was reported in 13.6% (12 patients), alcohol in 12.5% of patients (11 patients), gastro esophageal reflux disease in 14.6% of patients (13 patients) and voice abuse in

39.7% of patients (35 patients). The most common risk factor in vocal cord neoplasm is smoking, followed by voice abuse.

10. Prevalence of smoking in vocal cord lesions

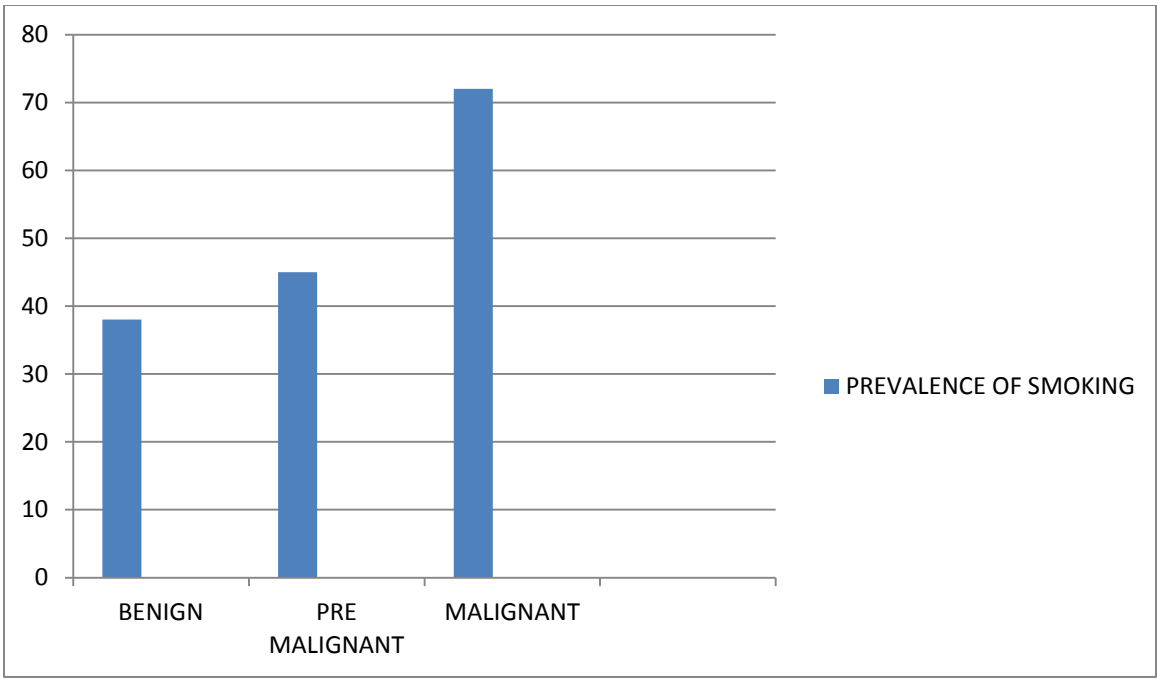


Fig.26 Prevalence of smoking in vocal cord lesions

History of smoking in present in 72% of patients with malignancy, 45% of patients with pre malignant lesions and only in 38% of patients with benign lesion. This shows a clear association of smoking in malignant lesions.

11. Prevalence of pan chewing in vocal cord lesions

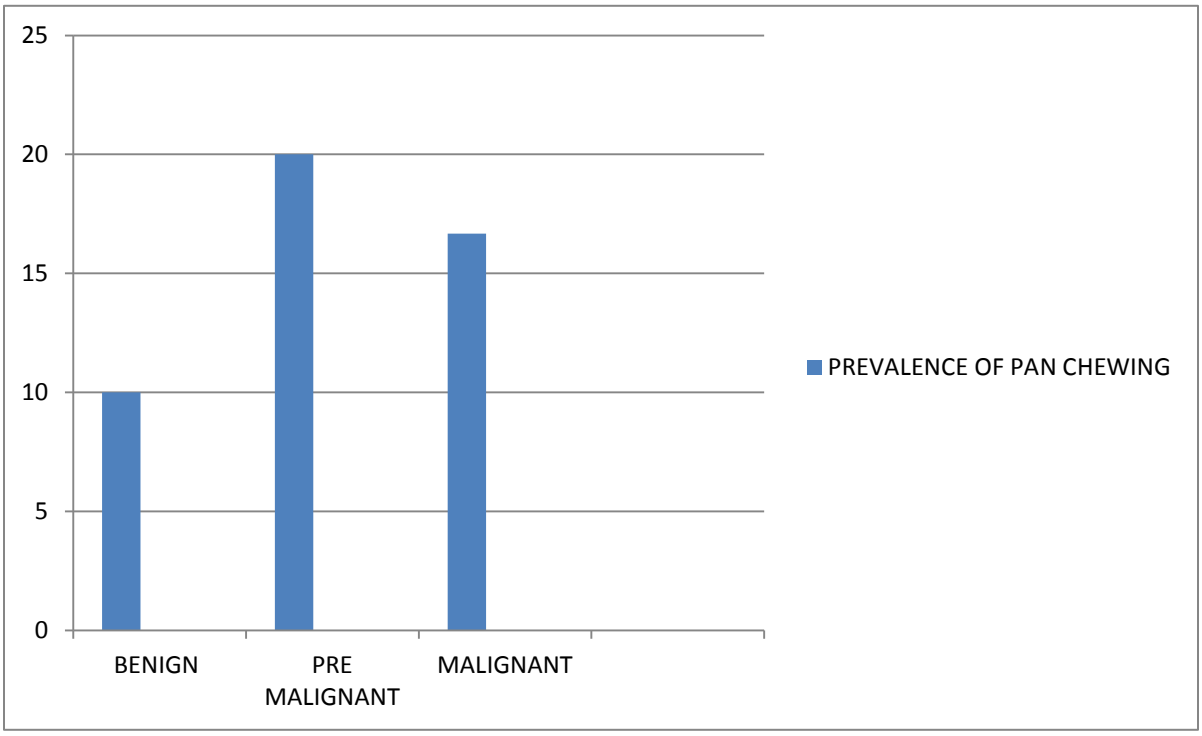


Fig.27 Prevalence of pan chewing in vocal cord lesions.

Pan chewing was reported in 16.6% of malignant lesions, 20% of pre malignant lesions and in 10% of benign lesions.

12. Prevalence of alcohol use in vocal cord lesions

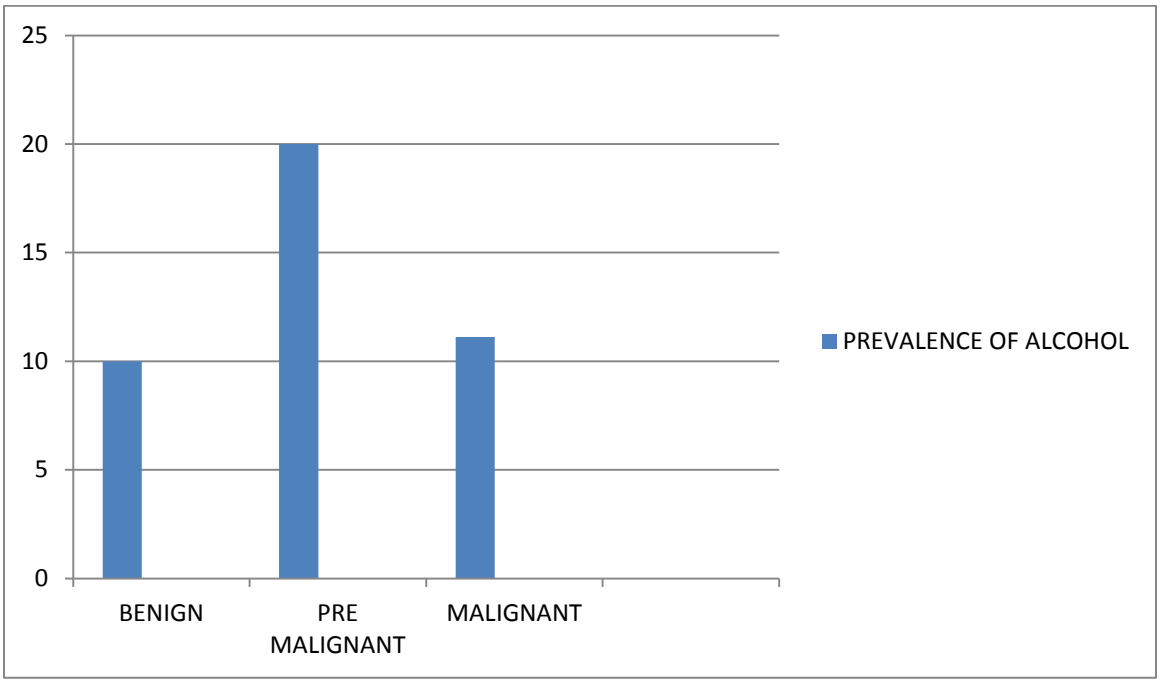


Fig.28 Prevalence of alcohol use in various vocal cord lesions.

History of alcohol use was present in 11.1% of malignant lesions, 20% of pre malignant lesions and in 10% of benign lesions.

13. Prevalence of Gastro oesophageal reflux disease in vocal cord lesions

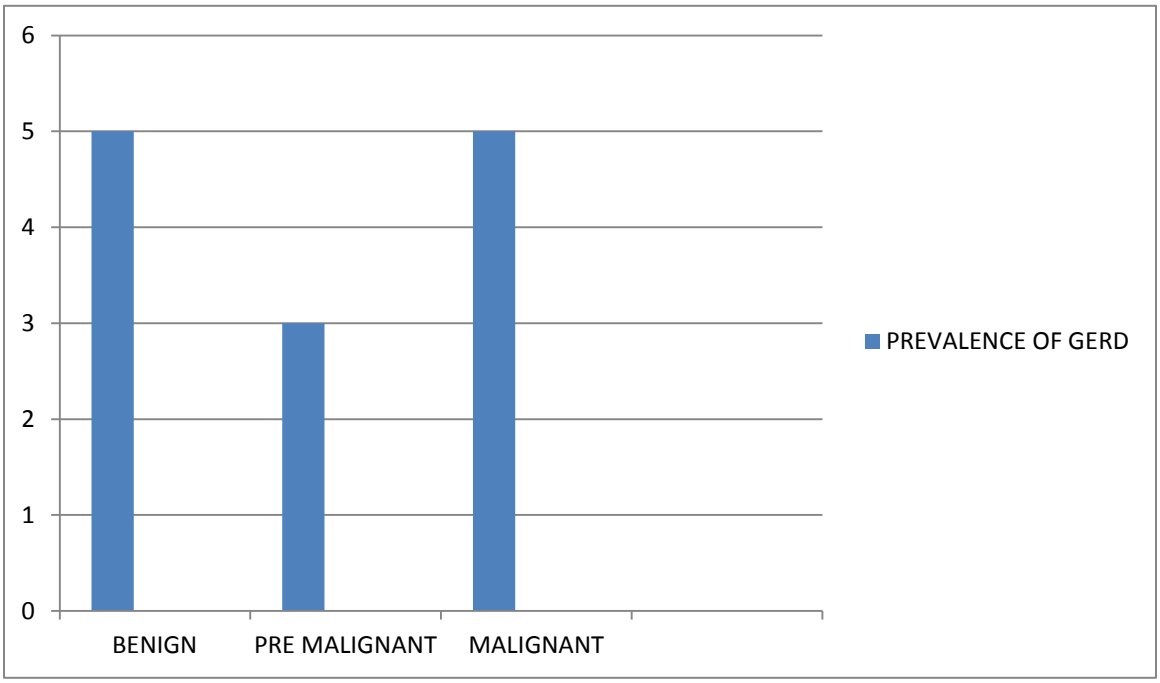


Fig.29 Prevalence of Gastro oesophageal reflux disease.

Gastro esophageal reflux disease is prevalent in 9% of benign lesions (5 patients), 10% of pre malignant lesions (3 patients), and in 27.7% of malignant lesions (5 patients). This indicates that there is a definite association between gastro oesophageal reflux disease and vocal cord pathology.

14. Prevalence of voice abuse in vocal cord lesions.

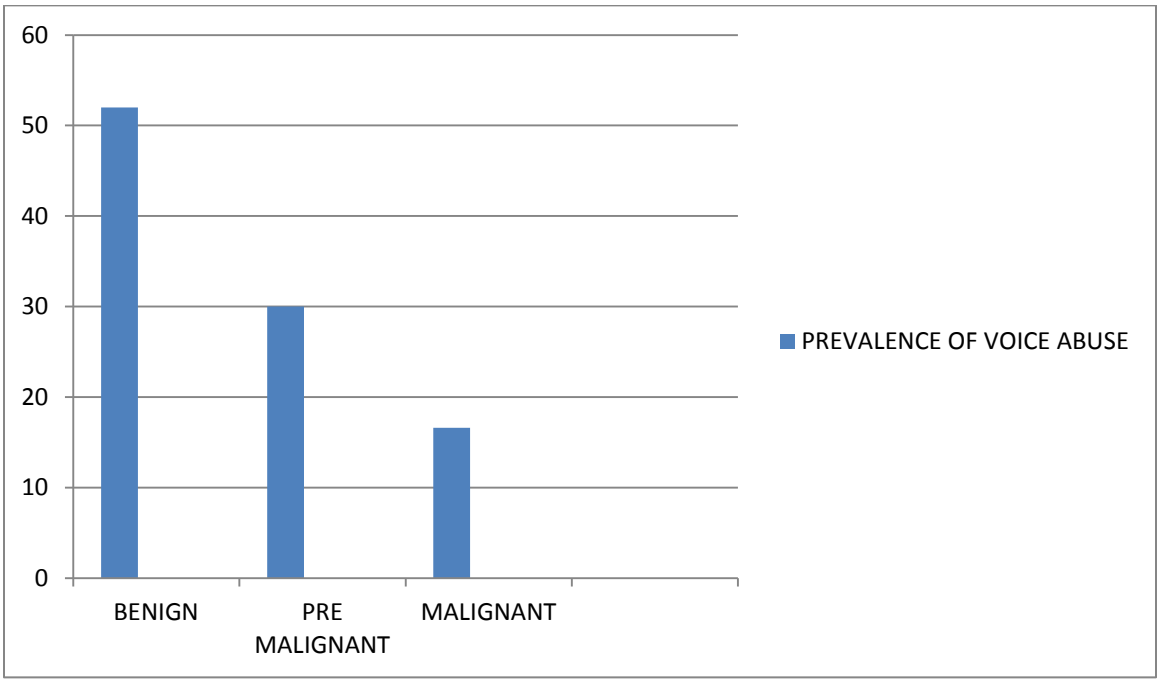


Fig. 30 Prevalence of voice abuse.

Voice abuse is reported in 52% of benign lesions (26 patients), 30% of pre malignant lesion (6 patients) and in 16.6% of malignant lesions (3 patients). This indicates that benign lesions have a definite association with voice abuse. Of the total 18 malignant cases, 2 patients (11% of patients) have only voice abuse as risk factor, In the case of pre malignant lesions of the total 30 patients 5 presented with voice abuse as the only risk factor.

15.Staining pattern of Lugol's iodine in various lesions.

A. Benign lesions

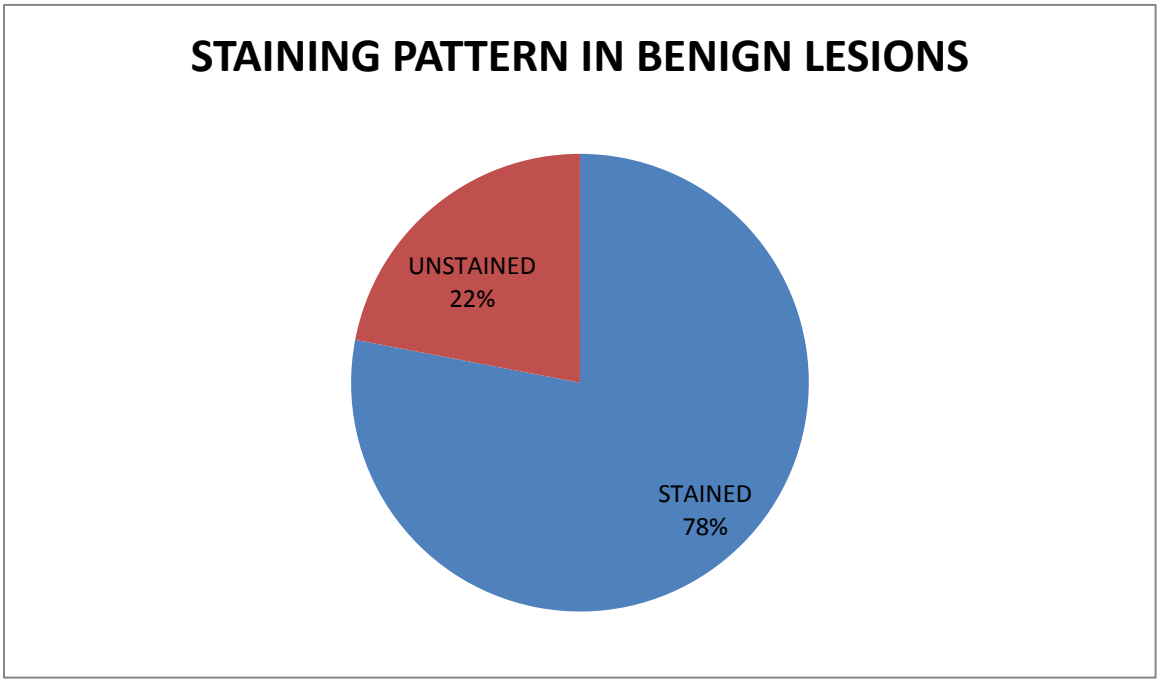


Fig.31 Staining pattern of Lugol's iodine in benign lesions.

Of the total 55 benign lesions 78% showed staining with Lugol's iodine. This matches with the hypothesis that benign lesions should stain with Lugol's iodine.

B. Malignant lesions

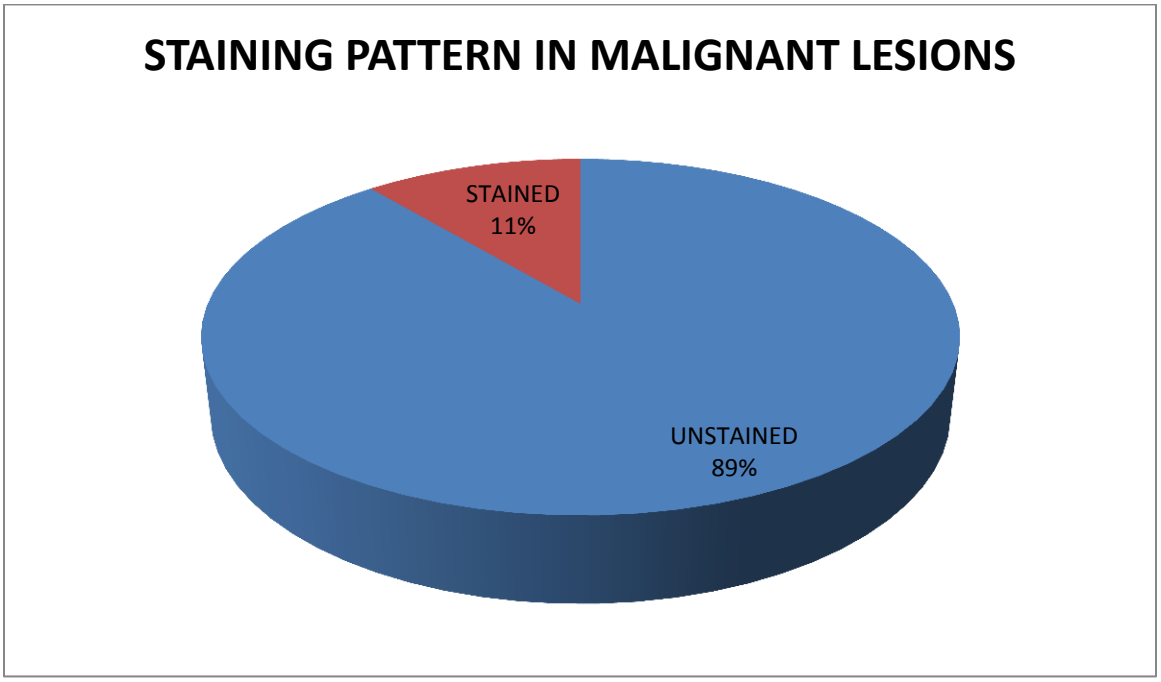


Fig.32 Staining pattern of Lugol's iodine in malignant lesions.

Of the total 18 malignant lesions (squamous cell carcinoma) 88.88% (16 patients) remained unstained. This matches with the hypothesis that malignant lesions remain unstained after application of Lugol's iodine.

C. Pre malignant lesions

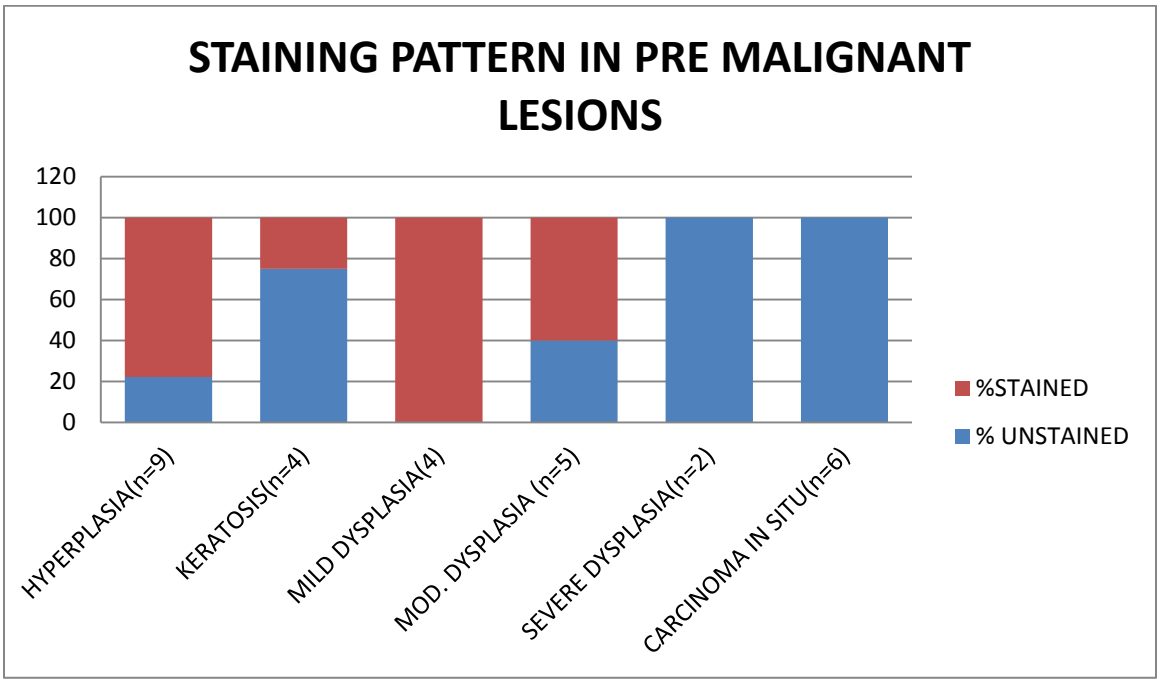


Fig.33 Staining pattern of Lugol's iodine in various pre malignant lesions.

As per the WHO classification of pre malignant lesions of vocal cords, the six groups of pre-malignant lesions were evaluated in our study (i.e. hyperplasia, keratosis, mild dysplasia, moderate dysplasia, severe dysplasia, and carcinoma insitu.). As we move high in the spectrum i.e. from hyperplasia to carcinoma in situ, the staining pattern changes from "stains" to "unstained". Of the 9 cases of hyperplasia, 7 cases stained and 2 cases remained unstained

(22.2% cases were unstained). In keratosis, of the total 4 cases, 1 case stained and 3 cases remained unstained (75% of cases were unstained). In mild dysplasia, of the 4 cases, all the 4 cases were stained brown during the application of Lugol's iodine (0% of cases were unstained). In moderate dysplasia, of the total 5 cases, 3 cases stained and 2 remained unstained (40% of cases were unstained). In severe dysplasia (2 cases) and carcinoma in situ (6 cases), all remained unstained after Lugol's iodine application. i.e. 100% lesions showed "unstained" pattern. This shows that in high risk cases i.e. severe dysplasia and carcinoma in situ, Lugol's iodine is an effective tool in identifying the lesion intra operatively.

16. **Sensitivity and specificity of Lugol's iodine in detecting squamous cell carcinoma**.

	MALIGNANT	PRE MALIGNANT	BENIGN	TOTAL
UNSTAINED	16	14	12	42
STAINED	2	16	43	61
TOTAL	18	30	55	103

Table.3 Staining pattern of Lugol's iodine in various vocal cord lesions.

1. Sensitivity of Lugol's iodine in detecting malignant lesions are calculated as

$\frac{16}{18}X100$= 88.88%

(95% confidence interval 65.29% - 98.62%)

2. Sensitivity of Lugol's iodine in detecting pre malignant lesions are calculated as

$$\frac{14}{30}X100 = 46.67\%$$

(95% confidence interval 28.34% - 65.67%)

3. Specificity of Lugol's iodine in detecting pre malignant or malignant lesions are

$$\frac{43}{55}X100 = 78.18\%$$

(95% confidence interval 64.99% - 88.19%)

17. Positive predictive and negative predictive value of Lugol's iodine in detecting malignant lesions:

	MALIGNANT	BENIGN	TOTAL
UNSTAINED	16	12	28
STAINED	2	43	45
TOTAL	18	55	73

Table.4 Staining pattern of Lugol's iodine benign and malignant vocal cord lesions

Positive predictive value for malignant lesions,

PPV $\frac{16}{28} X100 = 57.14\%$

(95% confidence interval 37.18%- 75.54%)

Negative predictive value for malignant lesions,

NPV $\frac{43}{45} X100 = 95.55\%$

(95% confidence interval 84.85%- 99.46%)

18. Positive predictive and negative predictive value of Lugol's iodine in detecting pre malignant lesions:

	PRE MALIGNANT	**BENIGN**	**TOTAL**
UNSTAINED	**14**	**12**	**26**
STAINED	**16**	**43**	**59**
TOTAL	**30**	**55**	**85**

Table.5 Staining pattern of Lugol's iodine in premalignant and benign vocal cord lesions

Positive predictive value for pre malignant lesions,

PPV $\frac{14}{26}$X100 = 53.85%

(95% confidence interval 33.37%- 73.41%)

Negative predictive value for pre malignant lesions,

NPV 43/59 X100 = 72.88%

(95% confidence interval 59.73% - 83.64%)

DISCUSSION

Cancer of the larynx is the second most common malignancy of the upper aerodigestive tract (UADT) [1]. This accounts for approximately 1.7% of all new cancer diagnosis, 25% of all head and neck malignancies and in 90% of cases is squamous cell carcinoma[2.] In India, laryngeal carcinoma constitutes 2.63% of all body cancers. It is ten times more common in males than females (4.79% vs 0.47%) [9.] Smoking, alcohol, chemical exposure, laryngopharyngeal reflux, viral etiology and diet are considered as the risk factors associated with laryngeal cancer. Squamous cell carcinoma of the larynx is strongly associated with the use of tobacco and alcohol. They are the two strongest aetiological factors for the development of Head and Neck Squamous Cell Carcinoma (HNSCC), both independently and synergistically [12,13.] This has been the same in our study too. The most common site for laryngeal squamous cell carcinoma is the glottic larynx [3].

90% of laryngeal carcinomas are developing from pre malignant lesions [33]. The diagnosis of a pre malignant lesion of the larynx must be based on the histological characteristics of the lesion[34]. The World Health Organization (WHO) classifies [29] pre malignant laryngeal lesions into six groups as Hyperplasia, Keratosis, Mild dysplasia, Moderate dysplasia, Severe dysplasia, and Carcinoma in situ (CIS). All premalignant lesions have the potential to transform to carcinoma. Lesions showing mild dysplasia and even those without dysplasia may progress into invasive cancer [5, 38, 39,40,41,42.] So it is essential to identify all premalignant lesions early. A long term follow up of all pre malignant lesions is warranted as they may develop into carcinoma even after many years [43.]

The visual appearance of a premalignant laryngeal lesion does not predict its histologic nature. Pre operative investigations like videostroboscopy also do not reliably differentiate premalignant from malignant lesions [57.] Biopsy is the gold standard for diagnosis and adequate sampling of the suspected area is important for the management. Various adjunctive techniques have been developed to improve the clinician's ability to characterise these lesions, to guide biopsies and to aid in resection of the vocal cord lesions intra operatively using microlaryngoscopic techniques or by laser resection.

The various techniques used for detection of pre malignant and malignant lesions are the use of vital dyes like toluidine blue and methylene blue, contact endoscopy, autofluorescence endoscopy, induced autofluorescence using 5-aminolevulinic acid (5-ALA), compact endoscopy, optical coherence tomography (OCT).

Lugol's iodine in the chromoendoscopic studies in oesophagus [71] is now being accepted as a reliable screening tool in peripheral and less equipped settings. In our study of the total 89 patients, 92% (82 patients) are males and 8% (7 patients) are females. There is an evident male preponderance for vocal cord disorders in all the three category i.e. benign, pre malignant and malignant lesions. In case of malignant lesions, our study shows a male to female ratio 17:1, which shows a higher population of male patients affected with laryngeal carcinoma comparing the study by Babak Saedi et al [7], which shows the male to female ratio is 9.5:1. In worldwide also the overall male-to-female ratio is about 4:1 to 10:1 as evidenced by various studies [8],[77].

In a study by Muhammad Rashid Zia et al[78] the mean age for presentation of laryngeal malignancy is 54, which is comparable with our study which reported the mean age as 52 years and 84% patients are in the middle aged populations (30-60years). 56% patients presented with

hoarseness of voice of less than 6 months duration, this matches with the classical teaching that vocal cord lesions present early.

In our study, in those patients who presented with hoarseness of voice, the distribution of vocal cord lesions are as follows: 57% of patients presented with benign lesions, 23% presented with premalignant lesions and 20% presented with carcinoma. This finding contradicts the study by Iseh KR et al, which showed that 66.7% of the vocal cord lesions are malignant and 33.3% are benign lesions[79]. No studies clearly mention the distribution of pre malignant lesions.

Regarding distribution of pre malignant lesions in the glottis, our study shows the following results in decreasing order: Hyperplasia 30%, Carcinoma in situ 20%, Moderate dysplasia 17%, Keratosis 13%, Mild dysplasia 13%, and Severe dysplasia 7%, which shows hyperplasia is the most common premalignant condition in vocal cords followed by carcinoma in situ. No studies have clearly reported the distribution of pre malignant lesions before.

Our study shows that 84% of patients who presented with hoarseness of voice have single cord lesions and 16% have bilateral lesions, i.e. 1 in every 5 patient had bilateral vocal cord lesions. This is in contrast to the results obtained by Swapan K Ghosh et al[80] who reported the ratio of unilateral and bilateral affection was near about 5: 4.

In our study other than hoarseness of voice which is present in all the patients, the other symptoms at presentation in the decreasing order are globus pharyngeus, noisy breathing, dysphagia, odynophagia, neck swelling, bilateral ear pain and haemoptysis. Next to hoarseness of voice, globus pharyngeus is the most common symptom. On comparing the study by Muhammad Rashid Zia et al [76] our study agree in the symptomatology of laryngeal malignancy.

The various risk factors which are analysed in our study are smoking, pan chewing, alcohol intake, gastro esophageal reflux disease, and voice abuse. Smoking was reported in 46.59% of patients and it is the most common risk factor in vocal cord lesions. Others in the decreasing order are voice abuse in 39.7% of patients, gastro esophageal reflux disease in 14.6% of patients, pan chewing in 13.6% and alcohol in 12.5% of patients. While analysing smoking alone in the study, history of smoking was present in 72% of patients with malignancy and 45% of patients with pre malignant lesions and only in 38% of patients with benign lesion. This shows a clear association of smoking with malignant lesions. History of alcohol use was present in 11.1% of patients with malignant lesions, 20% of pre malignant lesions and in 10% of benign lesions. In previous studies [12, 13, 14] it is reported that alcohol act synergistically with smoking and has a definite role in carcinogenesis, our study shows only 11.1% of patients with malignant lesions have alcohol use.

Pan chewing was reported in 16.6% of malignant lesions, 20% of pre malignant lesions and in 10% of benign lesions. We strongly suspect the role of pan chewing in laryngeal carcinoma. This correlates with the finding by Sankaranarayanan et al[81] who reported that a significant predisposing effect was observed of occasional pan chewing in laryngeal carcinoma.

Our study shows that gastro oesophageal reflux disease (GERD) is prevalent in 9% of benign lesions, 10% of pre malignant lesions and in 27.7% of malignant lesions. In comparison to the study by Galli et al [16] which reports 81% of patients with laryngeal carcinoma showed abnormal acid reflux on 24 hr pH testing, our study shows only 27.7% of patient with acid reflux.

Voice abuse is reported in 52% of benign lesions, 30% of pre malignant lesion and in 16.6% of malignant lesion. Even though voice abuse is a well known risk factor for benign lesion

as reported by Swapan K Ghosh et al, [78] which matches with our results for benign lesions, our study also shows that 16.6% of patients with pre malignant lesions and 11% patients with carcinoma presented with voice abuse as the only risk factor. This reveals the role of voice abuse as a single risk factor and thus indicates the need for further studies to find out the prevalence of vocal abuse in pre malignant and malignant lesions.

Regarding the staining pattern of Lugol's iodine, in benign lesion it stained 78% of lesions and remained unstained in 88.88% of malignant lesions. This matches with the hypothesis that "Lugol's iodine stains the benign lesions and malignant lesions remain unstained. In addition to that it was noticed that after application of the dye, the lesions became well defined from the surrounding normal mucosa and in a few cases it was noticed that, the lesion appeared more extensive than prior to application of the dye. This study is first of its kind wherein the application of Lugol's iodine in the diagnosis of malignant neoplasm of the vocal cords has been studied. It concurs with the report of its use in other UADT malignancies.

As per the WHO classification of pre malignant lesions of vocal cords, the six groups of pre malignant lesions were evaluated in our study (i.e. hyperplasia, keratosis, mild dysplasia, moderate dysplasia, severe dysplasia, and carcinoma insitu). As we move high in the spectrum from hyperplasia to carcinoma in situ, the staining pattern changes from "stains" to "unstained". In hyperplasia, 22.2% cases were unstained, in keratosis, 75% of cases were unstained, in mild dysplasia none of the cases were unstained, in moderate dysplasia, 40% of cases were unstained, where as in severe dysplasia and carcinoma in situ, all the cases remained unstained after Lugol's iodine application. This shows that in high risk cases i.e. severe dysplasia and carcinoma in situ, Lugol's iodine is an effective tool in identifying the lesion intra operatively.

In this study we found that, sensitivity of Lugol's iodine in detecting malignant lesions is 88.88% and sensitivity of Lugol's iodine in detecting pre malignant lesions is46.67%. At the same time the Specificity of Lugol's iodine in detecting pre malignant or malignant lesions is 78.18%.

According to Lundgren and colleagues [59], toluidine blue can detect dysplasia or malignant changes with 91% sensitivity, but only 52% specificity. Comparing with this, Lugol's iodine has a low sensitivity (91% Vs46.67%), but has a high specificity (52% Vs 78%) in detecting premalignant lesions.

In a study by Mehlmann et al, [62] in patients with suspected or proven laryngeal malignancy 5-ALA–induced fluorescence endoscopy demonstrated a sensitivity of 95% and a specificity of 80%, comparing to Lugol's iodine this study has only a marginal advantage in sensitivity (95% Vs 88.88%) and specificity (80% Vs 78.18%). But this had disadvantages like Autofluorescence was unreliable when scarring was present, whereas 5-ALA–induced fluorescence resulted in false-positive findings in inflamed lesions[82, 63].

Even though Lugol's iodine is a vital dye and it has been used in the lesions of the oral cavity, oropharynx and oesophagus, no studies have investigated its use in vocal cord lesions. McMahon J et al[70] used Lugol's iodine in the resection of oral and oropharyngeal squamous cell carcinoma. They suggest that Lugol's iodine is a simple, inexpensive, and apparently effective means of obtaining surgical margins in the resection of oral and oropharyngeal squamous cell carcinoma.

Massimo and colleagues[6] have done a review of various studies on the use of Lugol's iodine in oral cancer diagnosis. They found that Lugol's iodine has a sensitivity of 0.875 and specificity of 0.842 in detecting oral squamous cell carcinoma. They also noted that Lugol's solution has less sensitivity in identifying oral malignant and dysplastic disease but it was of greater specificity. Our study also detect similar findings, for malignant lesions Lugol's iodine has a sensitivity of 88.88% and specificity of 78.1%, whereas in dysplastic lesions it shows low sensitivity (46.67%) but high specificity (78.18%).

In a study by Stanford and colleagues[74], during oesophageal endoscopy, after staining with Lugol's iodine, the sensitivity of USL (Un Stained Lesions) for identifying high grade dysplasia or carcinoma was 96% and the specificity was 63%. They also noted that after staining with Lugol's iodine the lesions were larger or more clearly defined. Our study shows a sensitivity which is slightly less (88.8%), but with a high specificity (78.18%).

CONCLUSION

Lugol's iodine is a vital dye which has been used in the lesions of the oral cavity, oropharynx and oesophagus. However no studies have investigated its use in vocal cord lesions. Our study evaluated the staining property of 1.5% of Lugol's iodine in various vocal cord lesions and it analysed the efficacy of Lugol's iodine in detecting vocal cord pre malignant and malignant lesions. It stains the normal mucosa and benign lesions to brown or mahogany brown where as malignant lesions remain unstained. In various pre malignant lesions as it goes from epithelial hyperplasia to carcinoma in situ in the spectrum of pre malignant lesions, the staining pattern changes from normally stained to unstained lesions, and in case of severe dysplasia and carcinoma in situ it showed 100% unstained pattern. Lugol's iodine has a high sensitivity (89%) and specificity (79%) in detecting malignant lesions. In case of pre malignant lesions it has a sensitivity of 47%, but specificity of 78% altogether. As this is the first study, which assesses the staining pattern of Lugol's iodine in normal and diseased vocal cord, further studies are needed to find out its various applications including resection of vocal cord lesions and assessing the margin of the lesions.

Hence in conclusion we suggest the routine use of Lugol's iodine as a screening tool for ruling out pre-malignant and malignant lesions in all suspected vocal cord lesions undergoing microlaryngoscopic evaluation and resection. This commonly available, inexpensive, easily applicable and repeatable, cost effective test would be able to detect the odd non-keratotic lesion as malignancy or premalignant lesion thus facilitating complete resection.

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APPENDIX

PATIENT'S CONSENT FORM

STUDY: Diagnostic reliability of Lugol's iodine as an adjunctive tool in the evaluation of laryngeal neoplasm.

Study Number :

Participant's Name :

Hospital number :

Date of birth / Age :

Please tick (√) each box if you have read the study information sheet telling you about the study and you have understood what the doctor have told you.

I have read and understood about the study [ ]

I have been able to ask any questions [ ]

I understand that - I do not have to take part in the study and I can stop at any time [ ]

I understand that - I do not have to give a reason for wanting to stop taking part [ ]

I understand that - if I stop participating , it will not affect my treatment [ ]

The doctors who are involved in the study can get information from my chart at any time for this study [ ]

Later, if they need to do some more research using my data they can also get other information from my chart for that [ ]

I understand that my name will be not given or shown to anybody not involved in the study [ ]

I agree not to stop any data or results being used from this study as long as it is for scientific reasons [ ]

I agree that I can take part in the above study [ ]

Signature (or thumb impression) of the patient : Date :

Signatory's name :

Signature of the Investigator : Date :

Study investigator's Name :

Signature of the witness : Date :

Name of the witness :

PATIENT INFORMATION SHEET

You are requested to participate in this study which is aimed at finding out the effectiveness of Lugol's Iodine in detecting cancer of the larynx and vocal cord polyps. In this study, you will be asked some questions about your disease. If you are clinically diagnosed with cancer of the voice box or with vocal cord polyps or with any lesions of the vocal cords, a biopsy is advised to confirm the diagnosis. A microscope assisted visualization of the voice box is done and tissue is taken for biopsy under general anaesthesia after taking written valid consent. This is done routinely for all patients with similar diagnosis. During the process of taking tissue for biopsy, a dye named Lugol's Iodine is applied and staining is noted for analysis. There is no other risk involved in this study. Patient need not pay any extra money for the tests. This study can help in diagnosing and screening laryngeal cancer. You can withdraw from this study at any moment if you feel so, and that in no way will compromise your treatment at ENT department. Your participation in the study will remain confidential and shall be known only to the investigators. For any queries you can contact:

Dr VK Anand
PG Registrar
Dept. of ENT,
CMC Vellore
Mob: 09486840323.

PROFORMA

EFFICACY OF LUGOL'S IODINE IN THE EVALUATION OF VOCAL CORD NEOPLASM

Serial No.:

Name:

Age:

Sex:

Hospital number:

Date of Admission:

Unit:

Duration of hoarseness:

Any other complaints:

(Dysphagia/ Odynophagia/ breathing difficulty/ neck swelling etc)

Any previous treatment taken:

(surgery/ chemotherapy/ radiotherapy)

Addictions:

(smoking/ alcohol/ pan chewing/ GERD/ voice abuse)

NPL scopy findings:

Surgery date:

Video recording (yes/no):

MLscopy findings:

Stained / unstained:

Biopsy no. and date:

Biopsy report:

Final Diagnosis:

Category:

(1=benign, 2=pre malignant, 3=malignant, 4=bilateral lesions)

Data analysis sheet

serno	name	hospno	age	sex	date	durtn	othrcm	dyspha	neck	others	surgery	chemo	RT	tobacco	pan	alcohol	gerd	voice	others1	nplscopy	srgrydte	video	mlsfndngs	stain	biopsy	report	fnldgnss	catego
1	Goutam Bho	087747f	48	1	06/12/2011	6	NO	NO	NO		NO	NO	NO	YES	YES	NO	NO	NO			06/12/2011	NO	Polypoidal u	NO	39237/11	moderately c	carcinoma	3
2	Mukul Gorai	103899f	50	1	02/01/2012	2	NO	NO	NO	N	NO	NO	NO	NO	NO	NO	NO	YES	N		03/01/2012	NO	Large pedun	YES	202/12	Papillomatou	Benign poly	1
3	Gouranga G	059621f	50	1	04/01/2012	3	NO	NO	NO	Nil	NO	NO	NO	NO	NO	NO	NO	YES	nil	right vocal c	04/01/2012	NO	pale cyst on	NO	327/12	benign voca	Benign voca	1
4	Durga prasa	910646d	57	1	08/01/2012	0	NO	NO	NO	odynophagia	NO	NO	NO	NO	NO	NO	NO	NO	nil	small cyst on the superio		NO	Right vc ker	NO	823/12	Right vc-hyp	Right vc- ke	4
5	Gour bhanda	106789f	55	1	10/01/2012	24	NO	NO	NO	nil	NO	NO	NO	NO	NO	NO	NO	YES	nil	proliferative	10/01/2012	NO	proliferative	NO	1119/12	moderately c	carcinoma le	3
6	Uttam Pal	115347f	36	1	17/01/2012	2	NO	NO	NO	nil	NO	NO	NO	YES	NO	NO	NO	NO	nil	polyp on the	17/01/2012	NO	broad based	YES	1794/12	Benign voca	Benign voca	1
7	Satya naraya	078064f	51	1	23/01/2012	72	NO	NO	NO	nil	NO	NO	NO	YES	YES	NO	NO	YES	nil	slough cove	24/01/2012	NO	Pedunculate	YES	2713/12	benign polyp	Benign poly	1
8	Vughnuna	118473f	59	1	30/01/2012	3	NO	NO	NO	nil	NO	NO	NO	YES	NO	NO	NO	NO	nil	irregular gro	31/01/2012	NO	congested p	YES	3500/12	full thicknes	right vocal c	2
9	Priyom das	805349d	22	1	06/02/2012	72	NO	NO	NO	nil	NO	NO	NO	NO	NO	NO	NO	NO	nil	papilloma rig	06/02/2012	NO	large papillo	YES	4233/12	laryngeal pa	Recurrent la	1
10	Dayaram Bh	114412c	47	1	07/02/2012	3	NO	NO	NO	nil	NO	NO	NO	NO	NO	NO	NO	YES	nil	left vocal co	08/02/2012	YES	sessile polyp	YES	4408/12	benign polyp	benign polyp	1
11	Dilip saha	092886f	54	1	07/02/2012	24	NO	NO	NO	nil	NO	NO	NO	YES	NO	NO	NO	NO	nil	small keratot	08/02/2012	YES	keratotic pat	NO	4496/12	Right vc-dys	carcinoma in	4
12	Jyoshna kun	116899f	39	2	22/02/2012	24	NO	NO	NO	throat pain	NO	NO	NO	NO	NO	NO	NO	YES	nil	Rt vc-minima	22/02/2012	NO	irregular muc	YES	6071/12	benign polyp	Reinkes oed	4
13	Ajoy das	142893f	40	1	22/02/2012	12	NO	NO	NO	globus phary	NO	NO	NO	YES	NO	NO	NO	NO	nil	?keratosis ?l	22/02/2012	NO	keratotic mu	NO	6073/12	b/l keratosis	bilateral voc	4
14	Sekhar Dutta	862772d	47	1	09/03/2012	6	NO	NO	NO	nil	NO	NO	NO	NO	NO	NO	NO	NO	nil		12/03/2012	NO	small polyp (	NO	8408/12	benign polyp	benign polyp	1
15	Lal bahadur	181037f	46	1	18/04/2012	2	NO	NO	NO	globus phary	NO	NO	NO	NO	NO	NO	NO	NO	nil	polypoidal le	18/04/2012	NO	nodular grov	NO	12921/12	hyperplasia,	Right vocal (	2
16	Thomas mat	201442f	50	1	11/05/2012	5	NO	NO	NO	globus phary	NO	NO	NO	YES	NO	NO	NO	NO	nil	left vocal co	11/05/2012	NO	proliferative	NO	15725/12	moderately c	carcinoma	3
17	Tapath kuma	201179f	33	1	15/05/2012	3	NO	NO	NO	nil	NO	NO	NO	YES	NO	NO	NO	NO	nil	bilateral voc	16/05/2012	YES	keratotic pat	NO	16329/12	hyperkerato	bilateral voc	2
18	Natai das	197959f	42	1	21/05/2012	36	NO	NO	NO	occassional d	NO	NO	NO	YES	NO	NO	NO	NO	nil	small pedun	23/05/2012	NO	polyp on the	YES	17099/12	benign fibro	benign fibro	1
19	Basudev Go	199250f	63	1	21/05/2012	5	NO	NO	NO	nil	YES	NO	NO	YES	NO	NO	NO	NO	nil	irregular gro	22/05/2012	NO	papillomatou	YES	16969/12	bilateral squ	bilateral squ	4
20	kalipada das	193415f	54	1	29/05/2012	12	NO	NO	NO	nil	NO	NO	NO	YES	NO	NO	NO	NO	nil	exophytic gr	30/05/2012	NO	ulcerative gr	NO	18020/12	mod. to poo	carcinoma	4
21	Abhijith dey	202225f	27	1	03/06/2012	8	NO	NO	NO	nil	NO	NO	NO	YES	NO	NO	NO	NO	nil	Pedunculate	04/06/2012	NO	pedunculate	NO	18446/12	benign voca	benign voca	1
22	Debendra na	188520f	50	1	05/06/2012	12	NO	NO	NO	nil	NO	NO	NO	YES	NO	NO	NO	NO	nil	left vc heam	06/06/2012	NO	small vocal c	YES	19277/12	benign fibro	benign polyp	1
23	Md.ali akkas	216486f	58	1	06/06/2012	8	NO	NO	NO	GERD	NO	NO	NO	YES	YES	NO	NO	NO	nil	growth on ri	07/06/2012	NO	exophytic gr	NO	18987/12	mod to poor	carcinoma	4
24	sunny josep	090545f	47	1	10/06/2012	48	NO	NO	NO	nil	NO	NO	NO	NO	NO	NO	NO	YES	nil	mucosal irre	11/06/2012	YES	scarring on t	NO	19279/12	carcinoma in	carcinoma in	2
25	Sulekha pari	219989f	46	2	11/06/2012	3	NO	NO	NO	nil	NO	NO	NO	NO	NO	NO	NO	YES	nil	sessile polyp	11/06/2012	YES	a broad base	YES	19281/12	benign voca	benign voca	1
26	Raj kumar	659232c	48	1	12/06/2012	6	NO	NO	NO	nil	NO	NO	NO	NO	NO	NO	NO	YES	nil	right vocal c	13/06/2012	NO	pedunculate	YES	19597/12	benign fibro	benign polyp	1
27	Jagadish cha	198061f		1	12/06/2012		NO	NO	NO		NO	NO	NO	NO	NO	NO	NO	NO		right vocal c	13/06/2012	NO	broad based	NO	19560/12	benign voca	benign voca	1
28	Devanandan	225257f	54	1	17/06/2012	12	NO	NO	NO	nil	NO	NO	NO	YES	NO	YES	NO	NO	nil	proliferative	18/06/2012	NO	proliferative	YES	20272/12	mod.differen	carcinoma	3
29	Thameem an	540403c	52	1	18/06/2012	1	NO	NO	NO	nil	NO	NO	NO	YES	NO	NO	YES	NO	nil	haemorrhagi	19/06/2012	NO	left vocal co	NO	20331/12	benign laryn	left vocal co	1
30	Dilip Kangi	204574f	28	1	20/06/2012	6	NO	NO	NO	nil	NO	NO	NO	NO	NO	NO	NO	NO	nil	left vocal co	21/06/2012	NO	a small polyp	YES	20706/12	benign laryn	benign voca	1
31	Banchanidhi	199772f	51	1	20/06/2012	2	NO	NO	NO	nil	NO	NO	NO	NO	NO	NO	NO	NO	nil	Right vocal (	21/06/2012	NO	bilateral voc	YES		epithelial aca	vocal cord n	1
32	Lakshmi	215146f	31	2	24/06/2012	36	NO	NO	NO	nil	NO	NO	NO	NO	NO	NO	NO	NO	nil	right vocal c	25/06/2012	NO	a pale polyp	NO	21123/12	benign voca	benign polyp	1
33	Babu Isaac	752335b	43	1	01/07/2012	2	NO	NO	NO	nil	NO	NO	NO	YES	NO	NO	NO	NO	nil	bilateral anterior 2/3rd ker		NO	mild irregula	YES	21923/12	focal mild dy	mild dysplas	2
34	Raghu mond	239579f	34	1	05/07/2012	7	NO	NO	NO	nil	NO	NO	NO	NO	NO	NO	NO	NO	nil		06/07/2012	NO	broad based	YES	22560/12	fibro epitheli	benign left v	1
35	Nanni Goun	239597f	82	1	08/07/2012	6	NO	YES	YES	bilateral ear p	NO	NO	NO	YES	NO	NO	NO	NO	nil	minimal cong	09/07/2012	YES	ulceroprolife	NO	22807/12	mod. differe	carcinoma gl	3
36	suresh	199186f	29	1	10/07/2012	3	YES	NO	NO	nil	NO	NO	NO	NO	NO	NO	NO	NO	nil	broad based	10/07/2012	NO	broad based	NO	23103/12	benign fibro	benign fibro	1
37	subrato gho	241450f	46	1	10/07/2012	5	NO	NO	NO	haemoptysis	NO	NO	NO	YES	NO	NO	NO	NO	nil	small polyp	11/07/2012	YES	polyp arising	YES	23148/12	benign fibro	benign polyp	1
38	Sumitra devi	244908f	38	2	11/07/2012	12	NO	NO	NO	nil	NO	NO	NO	NO	NO	NO	NO	YES	nil	right vocal cord haemorrh		NO	polyp on the	YES	23299/12	benign fibro	benign polyp	1
39	MOHD.LIAI	071427F	57	3	31/05/2012	12	NO	NO	NO	N	NO	NO	NO	NO	NO	NO	NO	NO	N	30/04/12 - RI	01/06/2012	NO	THICKENEI	NO	18216/12	SQUAMOU	CARCINOM	3

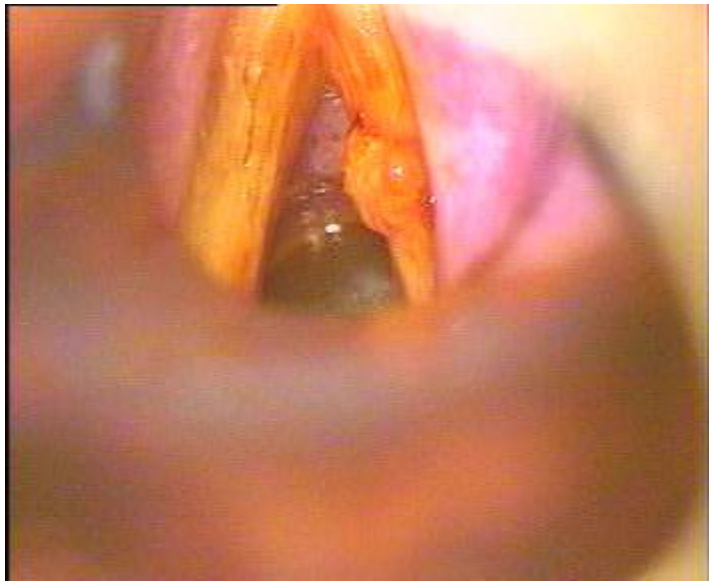
40	DIPANKAR	221623F	59	1	18/06/2012	8	NO	NO	NO	N	NO	NO	NO	YES	NO	NO	YES	NO	N	LEFT MONO	19/06/2012	NO	FIRM IRREG	NO	20329/12	MODERATI	MODERATI	2
41	Rama swamy	237008f	59	1	05/07/2012	36	NO	NO	NO	N	NO	NO	NO	YES	NO	NO	NO	NO	N	IRREGULAR	06/07/2012	NO	IRREGULAR	NO	22561/12	SQUAMOU	CARCINOM	3
42	DILIP MONI	238398F	50	1	12/07/2012	5	NO	NO	NO	N	NO	NO	NO	NO	NO	NO	NO	YES	N	POLYP ON T	13/07/2012	NO	SMALL HA	YES	23483/12	MILD EPITH	MILD DYSP	2
43	SAMAR MO	249546F	52	1	17/07/2012	4	YES	NO	NO	THROAT PA	NO	NO	NO	YES	NO	NO	NO	NO	N	PROLIFERA	18/07/2012	NO	INFILTRAT	NO	24171/12	SQUAMOU	CARCINOM	3
44	PRANAYES	198961F	57	1	01/08/2012	6	NO	NO	NO	N	NO	NO	NO	YES	NO	NO	NO	NO	N	BILATERAL	02/08/2012	NO	BILATERAL	YES	26029/12	MILD DYSP	MILD DYSP	2
45	BIVAS JAN	250542F	46	1	02/08/2012	6	NO	NO	NO	N	NO	NO	NO	YES	NO	YES	NO	NO	N	RIGHT VOC	03/08/2012	NO	POLYPS ON	YES	26274/12	BILATERAL	BILATERAL	4
46	NIRMAL SA	257819F	48	1	02/08/2012	12	YES	YES	NO	N	NO	NO	NO	YES	NO	YES	NO	NO	N	PROLIFERA	07/08/2012	NO	PROLIFERA	NO	26706/12	SQUAMOU	CARCINOM	3
47	UTPAL SAH	250494F	35	1	03/08/2012	18	NO	NO	NO	N	NO	NO	NO	YES	NO	NO	NO	YES	N	RIGHT VOC	03/08/2012	NO	BROAD BA	YES	26292/12	BENIGN PO	BENIGN PO	1
48	NEPAL BHU	228288F	34	1	05/08/2012	6	NO	NO	NO	N	NO	NO	NO	NO	NO	NO	NO	YES	N	RIGHT VOC	06/08/2012	NO	SMALL POI	YES	26382/12	BENIGN VO	BENIGN PO	1
49	ROY OOMM	262951F	53	1	07/08/2012	5	NO	NO	NO	N	NO	NO	NO	YES	NO	YES	NO	NO	N	WHITE IRRI	09/08/2012	NO	WHITE PAT	YES	26992/12	MODERATI	MODERATI	4
50	SHANMUGI	251563F	80	1	08/08/2012	1	NO	NO	NO	N	NO	NO	NO	NO	NO	NO	NO	NO	N	IRREGULAR	09/08/2012	NO	KERATOTIC	YES	26892/12	SQUAMOU	SQUAMOU	3
51	ABU CHAT	230236F	56	1	09/08/2012	3	NO	NO	NO	GLOBUS PHA	NO	NO	NO	NO	NO	NO	NO	YES	N	LEFT VOCA	10/08/2012	NO	PALE POLY	NO	27148/12	BENIGN PO	BENIGN PO	1
52	SUMANT K	248419F	42	1	10/08/2012	12	NO	NO	NO	N	NO	NO	NO	NO	NO	NO	NO	YES	N	PAPILLOMA	10/08/2012	NO	BROAD BA	YES	27158/12	BENIGN VO	LEFT VOCA	1
53	SONY BURI	922975D	35	1	10/08/2012	3	NO	NO	NO	N	NO	NO	NO	NO	NO	NO	NO	NO	N	LEFT VOCA	10/08/2012	NO	HAEMORRI	NO	27154/12	BENIGN VO	BENIGN PO	1
54	MALATHY	070001C	31	2	10/08/2012	4	NO	NO	NO	N	NO	NO	NO	NO	NO	NO	NO	YES	N	LEFT VOCA	10/08/2012	NO	PALE POLY	YES	27156/12	BENIGN VO	BENIGN PO	1
55	BIJALI BAL	261200F	62	2	12/08/2012	36	NO	YES	NO	N	NO	NO	NO	NO	YES	NO	NO	NO	N	ULCERATIV	13/08/2012	NO	ENTIRE LEN	NO	27287/12	CARCINOM	CARCINOM	3
56	SANJOY DE	177026F	36	1	22/08/2012	72	NO	NO	NO	N	NO	NO	NO	NO	NO	NO	YES	YES	N	KERATOSIS	23/08/2012	NO	KERATOTIC	YES	28654/12	BILATERAL	BENIGN PO	4
57	HARADSHA	276257F	62	1	23/08/2012	3	NO	NO	NO	N	NO	NO	NO	YES	NO	NO	NO	YES	N	PROLIFERA	24/08/2012	NO	PROLIFERA	NO	28813/12	MOD.DIFF.	CARCINOM	3
58	MAHADEV	255602F	61	1	26/08/2012	2	NO	NO	NO	N	NO	NO	NO	NO	NO	NO	NO	YES	N	RIGHT VOC	27/08/2012	NO	POLYPOIDA	YES	29008/12	EPITHELIAL	EPITHELIAL	2
59	Tapas Kuma	281656f	30	1	29/08/2012	2	NO	NO	NO	n	NO	NO	NO	NO	NO	NO	NO	YES	n	polyp on the	30/08/2012	NO	haemorrhagi	YES	29544/12	epithelial hy	Epithelial hy	2
60	Biswanath P	267921f	44	1	30/08/2012	4	NO	NO	NO	n	NO	NO	NO	NO	NO	NO	NO	NO	n	polyp on the	31/08/2012	NO	Haemorrhag	YES	29616/12	benign voca	Benign poly	1
61	Prasanjith Cl	282510f	36	1	30/08/2012	4	NO	NO	NO	n	NO	NO	NO	YES	YES	YES	NO	YES	n	Hemorrhagic	31/08/2012	NO	Large hemor	YES	29620/12	benign voca	Benign poly	1
62	Om Prakash	256097f	39	1	31/08/2012	48	NO	NO	NO	n	NO	NO	NO	NO	YES	NO	NO	NO	n	Right vocal c	31/08/2012	NO	cystic lesion	YES	29709/12	Right vocal c	Epithelial hy	2
63	Subrata bani	276927f	28	1	31/08/2012	9	NO	NO	NO	n	NO	NO	NO	YES	NO	YES	NO	NO	n	left vocal co	31/08/2012	YES	large polyp a	YES	29702/12	benign voca	Benign poly	1
64	Purushothar	908679c	49	1	04/09/2012	1	NO	NO	NO	n	NO	NO	NO	NO	NO	NO	NO	YES	n	Right vocal c	05/09/2012	NO	angiomatous	NO	30127/12	benign voca	Benign poly	1
65	sujay sardar	282594f	52	1	04/09/2012	6	NO	NO	NO	n	NO	NO	NO	YES	NO	NO	NO	NO	n	Irregular gro	05/09/2012	NO	ulceroprolife	NO	30233/12	mod.diff.squ	carcinoma rig	3
66	Bidhun Ranj	274334f	51	1	06/09/2012	12	NO	NO	NO	n	NO	NO	NO	NO	NO	NO	NO	NO	n	irregularity r	07/09/2012	NO	haemorrhagi	YES	30358/12	benign polyp	Benign poly	4
67	Bablu Hait	289934f	35	1	06/09/2012	3	NO	NO	NO	globus phary	NO	NO	NO	YES	YES	YES	NO	YES	n	Right vocal c	07/09/2012	NO	pale polyp o	YES	30482/12	benign voca	Benign poly	1
68	Mukul Saha	281861f	45	1	07/09/2012	12	NO	NO	NO	globus phary	NO	NO	NO	NO	NO	NO	NO	YES	n	left vocal co	07/09/2012	NO	hemorrhagic	YES	30573/12	benign voca	Benign poly	1
69	Sanatan Nas	260067f	58	1	07/09/2012	18	NO	NO	NO	n	NO	NO	NO	NO	NO	NO	NO	YES	n	left vocal co	07/09/2012	NO	reddish poly	YES	30579/12	vocal cord p	Benign poly	1
70	Venkata Ran	627327c	57	1	12/09/2012	1	NO	NO	NO	n	NO	NO	NO	NO	YES	YES	NO	NO	N	polyp on rig	13/09/2012	NO	small polyp c	YES	31292/12	right vocal c	Epithelial hy	2
71	Sk.Abdul ha	294002f	52	1	12/09/2012	36	NO	NO	NO	n	NO	NO	NO	YES	NO	NO	NO	NO	n	proliferative	12/09/2012	NO	ulceroprolife	NO	31133/12	squamous c	carcinoma gl	3
72	Md.Mehabu	278466f	45	1	16/09/2012	2	NO	NO	NO	n	NO	NO	NO	NO	NO	NO	NO	YES	n	left vocal co	17/09/2012	NO	pale polyp o	YES	31713/12	left vocal co	Mild dysplas	2
73	Ebrahim sk	293742f	33	1	18/09/2012	3	NO	NO	NO	globus phary	NO	NO	NO	YES	NO	NO	NO	NO	n	bilateral voc	18/09/2012	NO	polyp on RT	YES	31744/12	benign haen	Benign poly	4
74	Niranjan Das	253527f	54	1	18/09/2012	18	NO	NO	NO	globus phary	NO	NO	NO	NO	NO	NO	YES	YES	n	?left vocal c	18/09/2012	NO	whitish patc	YES	31741/12	left vocal co	Benign poly	1
75	Sabu Mathe	300270f	29	1	18/09/2012	3	NO	NO	NO	n	NO	NO	NO	NO	NO	NO	NO	YES	n	left vocal co	18/09/2012	NO	haemorrhagi	YES	31906/12	benign haen	Benign poly	1
76	Bankim Biha	302240f	58	1	19/09/2012	6	NO	NO	NO	n	NO	NO	NO	YES	NO	NO	NO	NO	n	white irregul	19/09/2012	NO	whitish patc	NO	31955/12	early invasiv	Carcinoma le	3
77	Sanjeet Rout	289432f	39	1	19/09/2012	7	NO	NO	NO	n	NO	NO	NO	NO	NO	NO	NO	NO	n	pedunculate	19/09/2012	NO	large friable	YES	31953/12	benign polyp	Benign poly	1
78	sanjoy Dutta	245466f	50	1	26/09/2012	18	NO	NO	NO	n	NO	NO	NO	YES	YES	YES	NO	NO	n	keratosis Rig	27/09/2012	NO	keratotic lesi	YES	33182/12	biopsy from	Moderate dy	2
79	Tapan kuma	307133f	39	1	27/09/2012	18	NO	NO	NO	n	NO	NO	NO	YES	NO	NO	NO	NO	n	polypoidal g	28/09/2012	NO	large haemo	YES		epithelial hy	Epithelial hy	2
80	kannan	299960f	65	1	27/09/2012	2	NO	NO	NO	n	NO	NO	NO	NO	NO	NO	NO	YES	n	proliferative	27/09/2012	NO	keratotic lesi	NO	33093/12	mod.diff.sq.c	Carcinoma ri	3
81	Hemanta Mo	293295f	36	1	28/09/2012	18	NO	NO	NO	n	NO	NO	NO	YES	YES	YES	NO	NO	n	sessile polyp	28/09/2012	NO	bilobed pedu	NO	33351/12	benign polyp	Benign poly	1
82	Jeeva	300992f	26	2	01/10/2012	6	NO	NO	NO	n	NO	NO	NO	NO	NO	NO	NO	YES	n	right VC-gro	02/10/2012	NO	proliferative	NO	33664/12	right and left	Carcinoma in	4
83	Jayanta Cho	315341f	31	1	09/10/2012	3	NO	NO	NO	n	NO	NO	NO	YES	NO	NO	NO	NO	n	right vocal c	09/10/2012	NO	whitish lesio	NO	34751/12	mild epitheli	Epithelial hy	2
84	Ramapada M	315893f	40	1	09/10/2012	3	NO	NO	NO	n	NO	NO	NO	NO	YES	NO	NO	YES	n	polypoidal le	09/10/2012	NO	a peduncula	YES	34743/12	benign voca	Benign poly	1
85	Santhi Pal	272816f	38	1	09/10/2012	48	NO	NO	NO	n	NO	NO	NO	NO	NO	NO	NO	YES	n	right vocal c	09/10/2012	NO	congested p	YES	34575/12	benign voca	Benign poly	1
86	Kripa Sindhu	284974f	42	1	09/10/2012	8	NO	NO	NO	n	NO	NO	NO	YES	NO	NO	NO	YES	n	right vocal c	09/10/2012	NO	right vocal c	YES	34579/12	acanthosis v	Benign poly	1
87	Dinesh Sil	314362f	49	1	14/10/2012	24	NO	NO	NO	n	NO	NO	NO	NO	NO	NO	NO	YES	n	irregular mas	15/10/2012	NO	broad based	YES	35375/12	benign voca	Benign poly	1
88	Iruday Doss	326396f	47	1	01/11/2012	6	NO	NO	NO	n	NO	NO	NO	NO	YES	YES	NO	NO	n	growth on le	02/11/2012	NO	keratotic pat	NO	37721/12	Rt-benign p	Rt.VC-Benig	4
89	Badarul Islar	231342f	33	1	12/11/2012	96	NO	NO	NO	globus phary	NO	NO	NO	YES	NO	NO	NO	YES	n	keratosis left	13/11/2012	NO	pale polyp w	YES	39002/12	benign voca	Benign poly	1

COLOUR PLATES

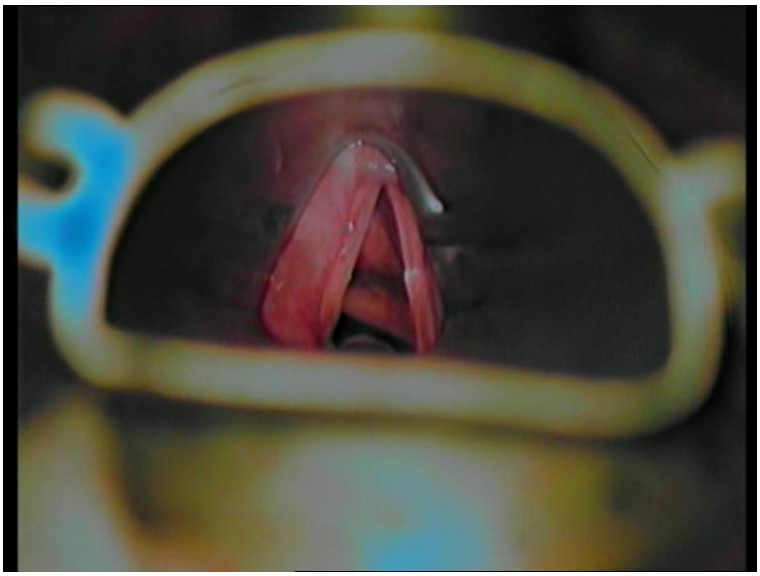
Colour plate. 1 Benign lesion on right vocal cord

Colour plate. 2 Wiping of the vocal cord with dry cotton ball

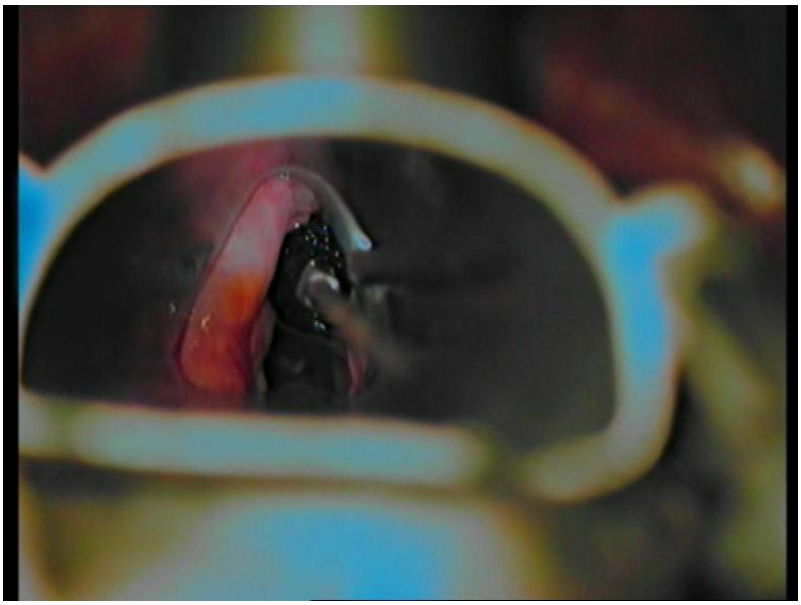
Colour plate. 3 Applying Lugol's iodine on the vocal cords



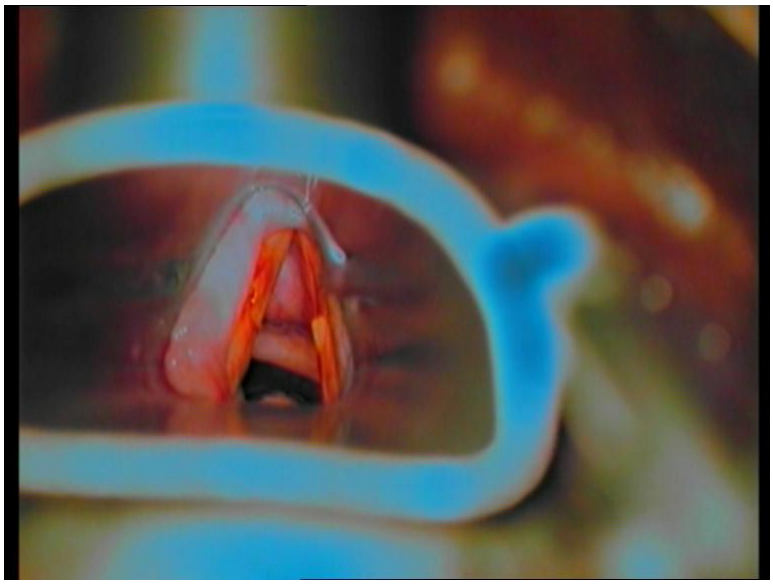
Colour plate. 4 Uniform staining of the benign lesion and vocal cords



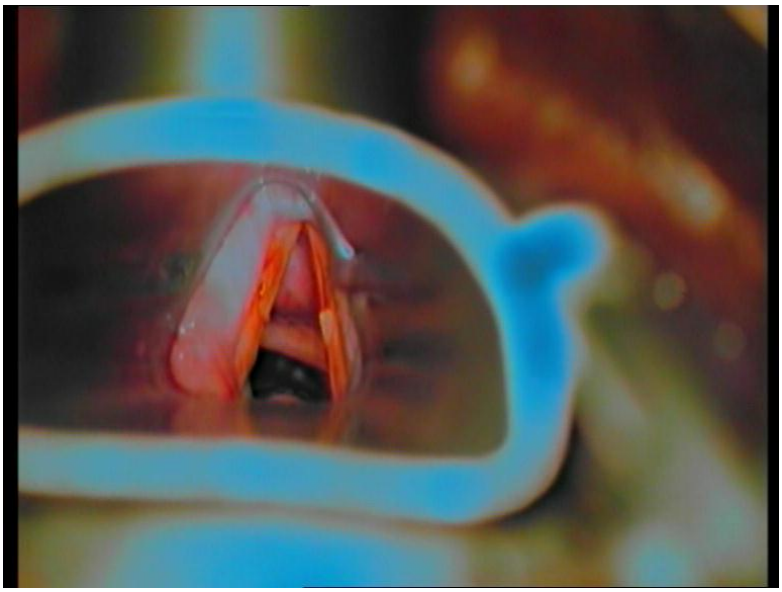
Colour plate. 5 High grade dysplasia on the right vocal cord



Colour plate. 6 Application of the Lugol's iodine on high grade dysplasia



Colour plate. 7 Immediately after the application of Lugol's iodine on high grade dysplasia



Colour plate. 8 Differential staining after 1 minute showing well defined lesion with clear margin of a high grade dysplasia

Colour plate. 9 Operating room set up during ML scopy.